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EXPERIMENTAL CHOLESTEROL ATHEROMATOSIS IN AN OMNIVOROUS ANIMAL, THE CHICK

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CHICAGO

Since cholesterol atherosclerosis was first produced in the rabbit [1] its relation to the problem of human arteriosclerosis has been in dispute. This relationship might be clarified if similar lesions could be produced in an animal more closely resembling man in its cholesterol metabolism than does the herbivorous rabbit. Attempts by numerous investigators to produce cholesterol atherosclerosis in omnivores and carnivores, such as the rat, mouse, dog and cat, have failed to yield consistent or clearcut results. Only in the herbivorous guinea pig have the lesions approximated those in the rabbit,[2] but lesions appear only microscopically.

The significance of the experiments in rabbits has been further questioned [3] on the ground that the experimentally produced atheroma does not resemble the spontaneous arterial lesion of the rabbit, which consists of medial degeneration with calcification and intimal fibrosis without atheroma. It seemed desirable, therefore, to find an omnivorous animal in which spontaneous atherosclerosis develops with aging and to attempt premature production of similar lesions by experimental means.

The class Aves was selected for study since many orders of birds are either omnivorous or carnivorous, birds of advanced age show spontaneous arteriosclerosis more closely simulating that of man than do any of the mammals, and previous work suggested that it might be possible to reproduce these changes experimentally. It was recognized

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1. Anitschkow, N., and Chalatow, S.: Centralbl. f. allg. Path. u. path. Anat. **24**:1, 1913.

2. Anitschkow, N.: Beitr. z. path. Anat. u. z. allg. Path. **70**:265, 1922. Bailey, C. H.: Proc. Soc. Exper. Biol. & Med. **13**:60, 1915.

3. Duff, G. L.: Arch. Path. **20**:81 and 259, 1935.

that the metabolic processes of birds differ in some respects from those of man—for example, in respect to nitrogen catabolism and the function of the pancreatic islets—but the similarities of the vascular lesions seemed sufficient to warrant investigation of this class.

The chicken, a member of the Galliformes (ground birds), was chosen because it is omnivorous, readily available and suitable for laboratory study. Ground fowl show atherosclerosis with advanced age.[4] and even as early as the end of the second year of life.[5] In one series, arterial changes were found in 75 per cent of fowl examined.[5] As in man, the vascular changes are more frequent in males than in females.[4a, b] While avian atherosclerosis is generally most pronounced in the ascending aorta and in the brachiocephalic arteries,[4a] in the chicken, pronounced lesions have been reported in the abdominal aorta.[5]

Normal chick arteries, although similar in their pathologic changes to the vessels of man, differ anatomically in a number of respects from human arteries. The aorta and the large elastic arteries of the root of the neck of the chick are richer in elastic tissue than the corresponding human vessels.[4a] The change to the muscular type of vessel occurs in the midabdominal aorta.[6] The intima of the avian aorta and other arteries is very thin, consisting merely of endothelium and a fine layer of acellular fibrous tissue.[4] Unlike the intima of adult human arteries, the chick intima lacks a subendothelial fibrous and a musculoelastic layer. Furthermore, there is no well demarcated, thick elastic sheet forming an internal elastic lamina in either the aorta or the smaller arteries. Such differences in structure might be expected to modify the form and extent of lesions without affecting the underlying process, since in all other respects avian and human blood vessels are similar.

Several reports have appeared on the induction of atheromatosis in birds by the feeding of cholesterol or cholesterol-containing substances. These investigations all suffer from the limitations that the age of the experimental birds was either unknown or admittedly advanced and that the number of experimental animals was generally small and the number of controls even fewer. Chicks were used in three investigations. In the most elaborate one,[5] a group of 9 cocks was fed 0.3 Gm. of cholesterol in oil daily for varying periods. In 4 cocks, fed from four to fourteen months, high grade intimal thickening of the abdominal aorta developed; less marked changes occurred in 3 birds, fed from three weeks to three months, while 2 birds, fed for two to three weeks, and 2 control chicks failed to show any intimal thickening. In another

4. (*a*) Fox, H., in Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933, chap. 6, p. 153; (*b*) Bull. New York Acad. Med. **15**:748, 1939. (*c*) Yamagiwa, K., and Adachi, O.: Verhandl. d. Japan. path. Gesellsch. **4**:55, 1914.

5. Uchiyama, T.: Virchows Arch. f. path. Anat. **277**:642, 1930.

6. Pfister, H. I. C.: J. Anat. **61**:213, 1927.

study [7] a small group of chicks and pigeons was fed on egg yolks for periods up to two and one-half years. In several of these birds atherosclerosis developed, while in a number fed on hydrous wool fat arterial lesions failed to develop. There is also reported the experimental production of atherosclerosis in a 40 year old parrot fed on egg yolks for three years.[8] While these reports are suggestive, the possibility of spontaneous arterial changes was not ruled out.

PROCEDURE

Twenty-four 1 day old White Rock cockerels were placed in a battery with a thermostatically controlled heating unit and with access to fresh water and feed.

The feed was a commercial chick starter mash containing not less than 18 per cent crude protein, 3.5 per cent crude fat, 51 per cent nitrogen-free extract and not more than 6 per cent crude fiber. It contained 1.4 per cent of calcium, 0.75 per cent of phosphorus and 93 parts per million of manganese. The combined vitamin A and provitamin A (carotene) content was 1,500 U. S. P. units per

TABLE 1.—*Feeding Plan of the Cholesterol Series of Chicks*

Chicks	Age of Chicks, Weeks	Composition of Feed
12	1 - 2	5% cholesterol in dry mash and 12.5 cc. of cottonseed oil per gram of cholesterol
12	2 - 3	10% cholesterol in dry mash, with no oil added
10	3 - 4	5% cholesterol in dry mash, with no oil added
10-8	4 - 6	5% cholesterol in dry mash and 5 cc. of cottonseed oil per gram of cholesterol
8	6 - 7	Control mash without cholesterol or cottonseed oil
8-3	7 -15	2.5% cholesterol in dry mash and 10 cc. of cottonseed oil per gram of cholesterol

hundred grams and the vitamin D content 80 A. O. A. C. units per hundred grams. The mash was prepared from corn, wheat bran, wheat standard middlings, corn gluten meal, meat scraps, alfalfa meal, soybean oil meal, pulverized oats, fish meal, dried skim milk, dried whey, ground limestone, salt, manganese sulfate and fish liver oil. To insure adequate fresh vitamin A, 2.25 Gm. of cod liver oil U. S. P. was added to each 100 Gm. of feed before the latter was placed in the feed bins.

At the age of 10 days the 24 chicks were divided into equal groups. Twelve were kept on the control mash diet; the rest were fed the control mash diet plus crystalline cholesterol (Wilson) suspended in cottonseed oil (Proctor & Gamble Puritan Oil). The cholesterol was added to the cottonseed oil and dissolved so far as possible, and then the dry mash was stirred in until a homogeneous preparation was obtained. The oil was used not only to aid solution but also as a vehicle to further absorption of the cholesterol from the intestinal tract. In the rabbit cholesterol in oil produces lesions earlier and in smaller quantities than does dry cholesterol.[3] After a short trial period on this diet, during which the cholesterol-fed chicks ingested less feed than the controls, appeared stunted in growth and showed matted greasy yellowish feathers, a dry mixture of choles-

7. Kawamura, R.: Neue Beiträge zur Morphologie und Physiologie der Cholesterinsteatose, Jena, Gustav Fischer, 1927. Yamaguchi, M., abstracted, Japan. J. M. Sc. **2**:143, 1925.

8. Anitschkow, N.: Verhandl. d. deutsch. path. Gesellsch. **20**:149, 1925.

terol in mash was substituted for a fortnight in an effort to improve food intake and accelerate growth. Since growth continued to be impaired, the feeding of the cholesterol-oil mixture was resumed. Further, in an attempt to improve food consumption and growth, the percentage of cholesterol added to the mash was varied. In the sixth week of the experiment, because the chicks appeared as though they might not survive, the cholesterol was entirely omitted from the mash for one week. Definite improvement in size and appearance took place during this time. The cholesterol-oil feeding was then resumed with a smaller percentage of cholesterol in the mash. Over the fourteen week period of the experiment these chicks received on the average a diet containing 5 per cent cholesterol (in terms of crystalline cholesterol in dry mash) during the first three weeks and 2.5 per cent cholesterol in the last nine weeks. Details of the dietary regimen are given in table 1. Fresh water and feed were provided daily.

The amount of feed consumed by each group of chicks was determined by adding a known amount of feed and weighing the residue twenty-four hours later, two or three days in succession. The birds were observed as to appearance and

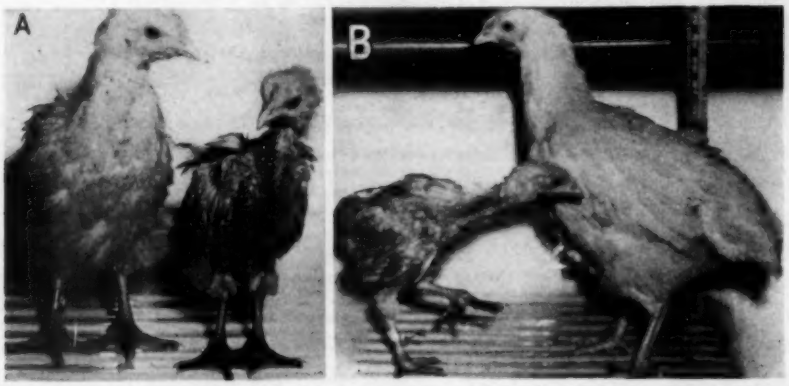

Fig. 1.—*A*, difference in size and in appearance of feathers between a control chick (left) and a cholesterol-fed one (right) at the age of 6 weeks. *B*, difference in appearance between a control chick (right) and a cholesterol-fed chick (left) at the age of 10 weeks.

behavior daily and were weighed weekly. Total cholesterol and cholesterol esters were determined by the Sperry-Schoenheimer method adapted for the photoelectric colorimeter [9] on blood samples obtained from the wing vein. No fasting preceded the drawing of blood for cholesterol and calcium determinations, but the blood sugar was determined after an eighteen hour fast. All chicks were autopsied, the survivors being put to death at the completion of the study. The organs were examined grossly, and sections for histologic study were prepared from the aorta, femoral arteries, heart, lungs, liver and kidney after fixation in solution of formaldehyde U. S. P. diluted 1:4. In some cases the pancreas, spleen, adrenal and testicle were likewise sectioned. The tissues were embedded in paraffin and stained with Delafield's hematoxylin and eosin. Sections of the aorta and left ventricle of the heart were likewise stained with the orcein elastic tissue stain, Masson connective tissue stain and with iron-hematoxylin and eosin. Frozen sections of the aorta, heart, lung, liver, kidney, spleen, testicle and colon were stained with sudan IV for lipoid.

9. Schoepheimer, R., and Sperry, W. M.: J. Biol. Chem. **106**:745, 1934.

RESULTS

The difference in development and appearance between the control and the cholesterol-fed cockerels was striking from the start (fig. 1). Not only were the cholesterol-fed chicks much smaller than the controls,

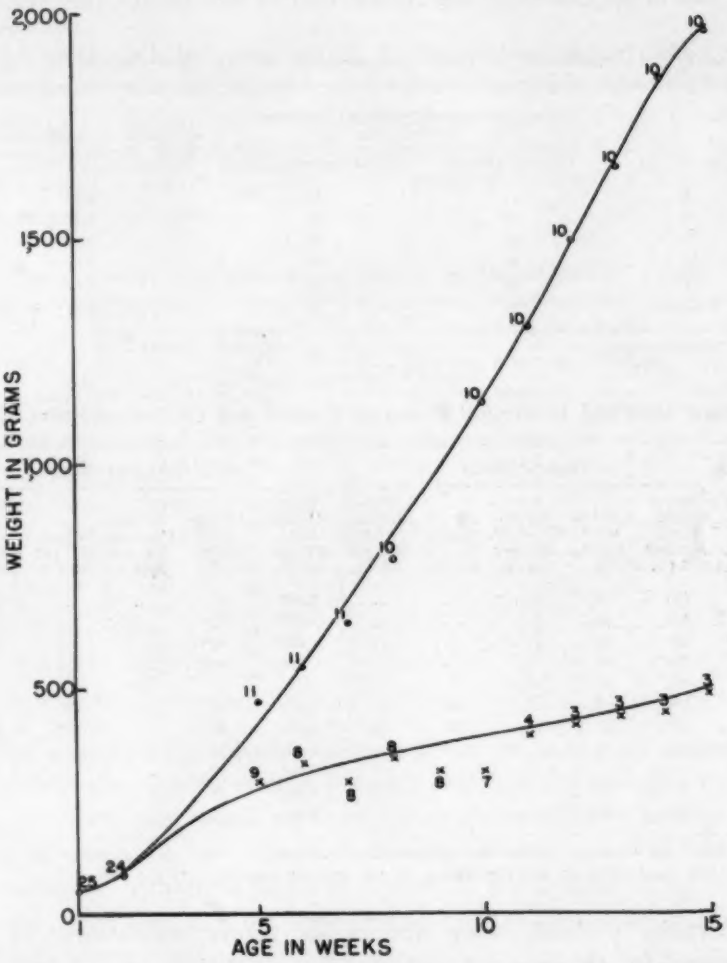

Fig. 2.—Growth curves of the two groups of chicks. Circles designate the average weights of the control birds; crosses, the average weights of the cholesterol-fed chicks. The figures placed along the two curves indicate the number of birds whose weights were averaged at each particular time.

but they also showed poor feather development and failed to acquire a mature comb, wattles or the crow of the cock. Cannibalism and pica were more marked than among the control birds. The difference in the growth curves is shown in figure 2. Their food consumption was one

fourth to one half that of the control chicks (table 2). Several deaths occurring in the early weeks of the experiment are attributed to infection with Salmonella pullorum since agglutination tests by the Illinois Department of Agriculture were positive for or suggestive of this infection in many members of the flock. Four deaths in the cholesterol-fed series and two in the control group are ascribed to this disease, the difference

TABLE 2.—*Comparison of Food Consumption in the Two Groups of Chicks*

Age, Weeks	Average Amount of Feed Consumed per Day, Gm.		Estimated Average Amount of Daily Cholesterol Intake of Cholesterol-Fed Chicks, Gm.
	Control Chicks	Cholesterol-Fed Chicks	
4	..	12	0.60
9	96	17	0.42
11	90	32	0.80
12	59	20	0.50
13	99	62*	1.55*
Average	86	29	0.78

* Probable error.

TABLE 3.—*Blood Cholesterol Levels in Control and Cholesterol-Fed Chicks*

Age, Weeks	Control Chicks					Cholesterol-Fed Chicks				
	Total Cholesterol, Mg. per 100 Cc.	Cholesterol Ester, %	Serum Calcium, Mg. per 100 Cc.	Blood Sugar, Mg. per 100 Cc.	Non-protein Nitrogen, Mg. per 100 Cc.	Total Cholesterol, Mg. per 100 Cc.	Cholesterol Ester, %	Serum Calcium, Mg. per 100 Cc.	Sugar, Mg. per 100 Cc.	Non-protein Nitrogen, Mg. per 100 Cc.
5	136	70	....	...	..	1,492	62	....	...	..
6	173	64	....	...	..	1,732	39	....	...	..
7	131	65	....	...	..	852*	65*	....	...	..
8	108	65	....	...	..	790†	67†	....	...	..
9	126	64	11.5	...	..	.....	..	11.6	...	..
10	108	65	....	150	..	1,432	40	11.6	261	..
11	100	63	....	187	34	1,980	63	....	179	28
12	113	66	....	...	..	3,464	71	....	...	..
						2,598	70	....	...	..
						2,944	73	....	...	..
13	88	60	....	...	..	1,344	74	....	...	..

* After one week on control feed (see feeding schedule).

† After four days on diet containing 2.5 per cent cholesterol.

in mortality probably being due to the poorer nourishment of the cholesterol-fed chicks.

The blood serum of the experimental chicks was grossly milky and opaque. The levels of total blood cholesterol were tremendously elevated over those of the controls (table 3). The ratio of cholesterol esters to total cholesterol at first dropped from 62 to 39 per cent under the influence of cholesterol feeding. When cholesterol feeding was interrupted for a week, as described, the total blood cholesterol fell while the percentage of esterified cholesterol returned to normal. On resumption of

cholesterol feeding, the percentage of esters dropped again as the total blood cholesterol rose; however, while the total cholesterol continued to rise, the ratio of esters stopped falling and rose to levels slightly above the initial and control values. The initial fall in percentage of cholesterol esters may indicate a period of initial liver lag, with later adaptation of the liver to the increased load of cholesterol and improved esterification.

The most striking gross feature in the cholesterol-fed chicks was the extreme fatty metamorphosis of the liver, confirmed microscopically with sudan IV stains. Interstitial accumulations of fat, either gross or microscopic, were also noted in the spleen, testis, colon, adrenals, heart and lymphoid tissue of the lung. The testes were much smaller than in the control cockerels.

TABLE 4.—*Age of Appearance of Lesions in Cholesterol-Fed Chicks*

Chick	Age in Days at Death	Number of Days of Cholesterol Feeding	Estimated Amount of Cholesterol Ingested in Grams	Lesions Found at Autopsy				
				Atheroma of Aorta	Atheroma of Brachio-Cephalic Arteries	Atheroma of Coronary Arteries	Atheroma of Splenic Arteries	Fatty Liver
1	20	12	18.6	0	0	0	0	0
2	20	12	18.6	0	0	0	0	0
3	33	22	28	0	0	0	0	+
4	41	30	36	0	0	0	0	++
5	60	42	48	+	0	0	0	0
6	67	49	55	0	0	0	0	++
7	67	49	55	++	++	+	0	+++
8	68	50	56	++	++	0	++	++
9	78	60	66	++	++	+++	++	+++
10	104	86	92	++	++	0	0	+++
11	105	87	93	++	++	0	0	+++
12	105	87	93	++	++	++	0	+++

The most striking vascular lesions were in the aorta, and resembled the earliest spontaneous arterial lesions.[4a] All 6 of the cockerels on the high cholesterol diet which survived ten or more weeks of feeding had gross atheroma of the aorta. A seventh showed only microscopic atheromatosis. Atheroma failed to develop in the 5 birds dying within the first eight weeks of the high cholesterol diet (table 4), and none of the 12 control birds showed lesions.

On gross inspection the affected aortas showed smooth, yellow-white, opaque, elevated, longitudinal plaques and granular longitudinal streaks. The plaques measured about 4 to 5 mm. in diameter and were irregular in outline. The greatest incidence of lesions was at the aortic arch and in the ascending aorta and about the orifices of the brachiocephalic arteries. Granular yellow streaks and small plaques were found in the

brachiocephalic arteries in every case in which the aorta was grossly involved. The aortas of the control chicks showed longitudinal wrinkling of the intimal surface of the ascending aorta but no lesions resembling those just described.

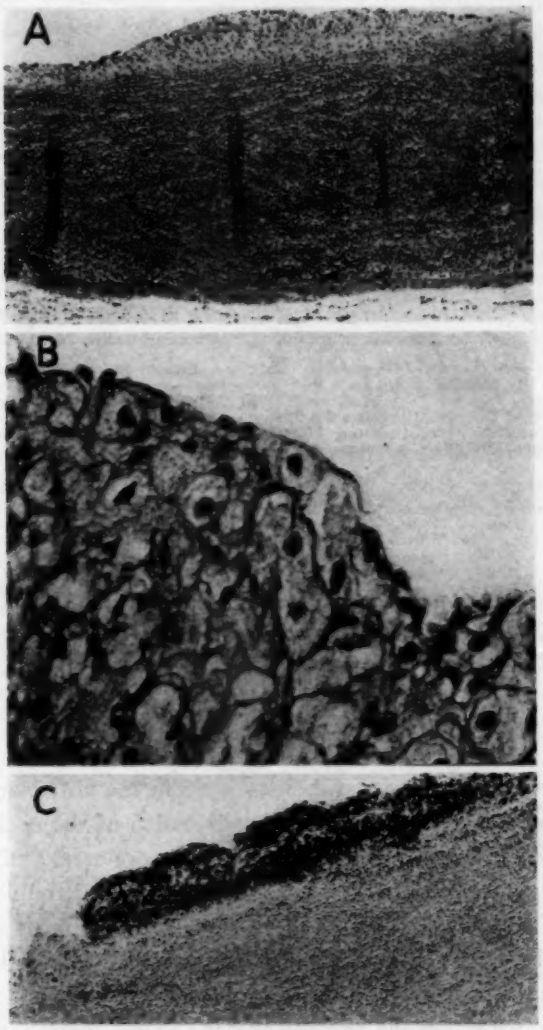

Fig. 3.—*A*, intimal plaque in a section of the aorta of a cholesterol-fed cockerel. Iron-hematoxylin stain; × 56. *B*, foam cells in an atheromatous plaque of cholesterol-fed chick. Hematoxylin-eosin stain; × 560. *C*, abundant fat-staining material in an intimal plaque of the aorta of a cholesterol-fed chick. Frozen section stained with sudan IV; × 89.

Microscopically, the plaques measured 50 to 75 microns in thickness and consisted of accumulations of large pale foam cells with small

pyknotic nuclei. The endothelium overlying these cell accumulations was intact, and the various stains revealed no changes in the media and adventitia (figs. 3 *A* and *B*). Fatty material in abundance was demonstrated within the subendothelial foam cells with sudan IV stains of

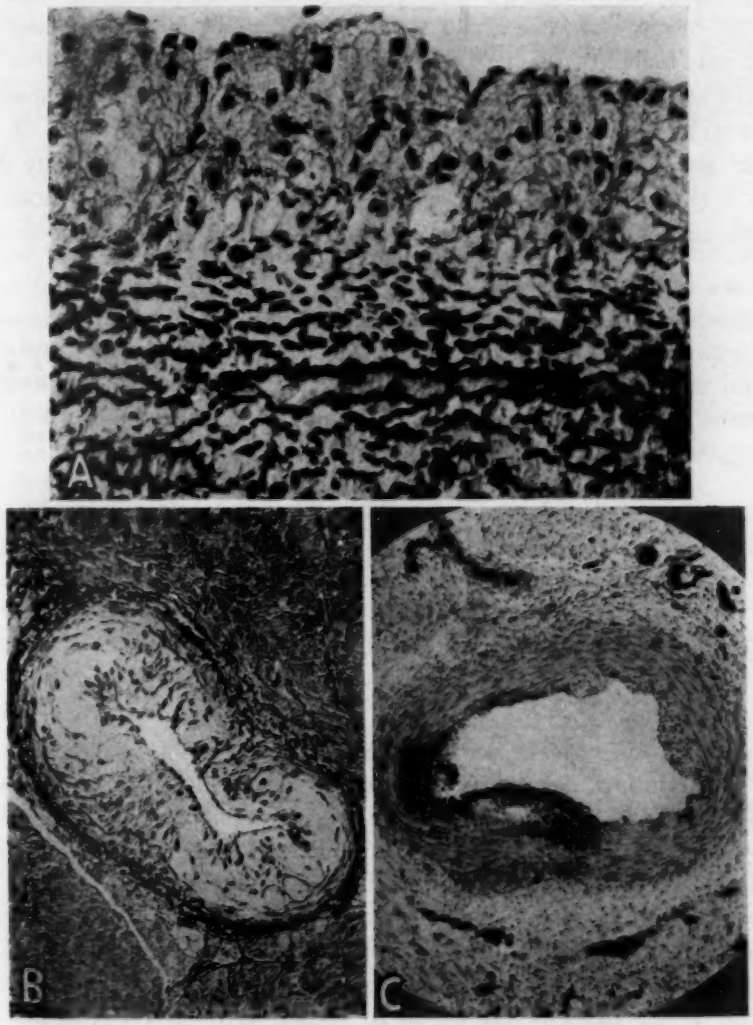

Fig. 4.—*A*, splitting of the innermost elastic fibers in the aorta of a cholesterol-fed chick, with fine wavy fibrils extending up into the intima. Orcein elastic tissue stain; × 480. *B*, tremendous intimal thickening, with narrowing of the lumen and compression of the media in a section of a coronary artery of a cholesterol-fed chick. Several large foam cells can be recognized in the intima. Masson connective tissue stain; × 150. *C*, lipoid material in an intimal plaque, with questionable extension into the media in a coronary artery of a cholesterol-fed chick. Frozen section, stained with sudan IV; × 80.

frozen sections (fig. 3 *C*), and doubly refractile rodlike crystals filling these cells were seen with the polarizing microscope. In some cases small extensions of fatty material penetrated into the media adjacent to the intima. Orcein stains demonstrated splitting of the elastica with fine, wavy elastic fibrils extending up between the foam cells adjacent to the media (fig. 4 *A*). No necrosis, ulceration, fibrosis or calcification was found.

In addition to the changes in the aorta and brachiocephalic arteries, atheromatosis was found microscopically in the small arteries of the heart and the spleen. While coronary lesions were found in only 3 of the 7 chicks showing aortic changes, it is likely that examination of additional sections would have disclosed lesions in the other birds with

TABLE 5.—*Relation of Heart Weight to Body Weight*

Series	Chick	Body Weight, Gm.	Heart Weight, Gm.	Ratio of Heart Weight to Body Weight, %
Control	1	1,816	9.7	0.53
	2	1,831	7.4	0.41
	3	2,000	10.4	0.52
	4	2,012	8.5	0.42
	5	2,041	9.1	0.44
	6	1,816	7.2	0.39
	7	1,906	8.2	0.42
	8	1,824	8.5	0.47
	9	2,131	9.7	0.46
	10	2,176	9.0	0.41
Cholesterol	10	784	5.2	0.66
	11	275	1.8	0.64
	12	450	3.7	0.81

aortic involvement. The coronary changes consisted of marked thickening of the intima and narrowing of the lumen, up to a slitlike aperture, and compression of the media with degeneration of the muscle fibers (fig. 4 *B*). In some instances the intimal thickening was localized and nodular, but often it involved the entire circumference (fig. 4 *B* and *C*). Large pale foam cells containing fatty material were found in the thickened intima (fig. 4 *C*), and at times the fatty deposits appeared to penetrate into the media, but the absence of a well demarcated internal elastic lamina made it difficult to be certain of this. The endothelium was intact throughout.

In 2 of the chicks with aortic involvement the small arteries of the spleen showed pathologic changes. The thickened intima contained extensive accumulations of a pale homogeneous material which stained with sudan IV.

Intraluminal fat was seen with sudan IV stains in the vessels of all organs, but examination failed to disclose any atheroma in the pulmonary, renal or femoral arteries or small vessels of other organs or in the veins.

The wet heart weight was measured for each of the control chicks and for each of the 3 chicks that survived longest in the cholesterol series, by the technic employed in this laboratory for the rabbit.[10] The results are shown in table 5. While the series is small, it may be stated that within this cholesterol-fed group the heart weight was higher in proportion to body weight than among the controls.

COMMENT

Our results indicate that atheromatous vascular lesions can be produced readily and quickly in the chick. In this particular omnivore, unlike some others, cholesterol feeding leads to tremendous increases in blood cholesterol and cholesterol esters and to fatty infiltration of many tissues, including the arteries. In the experimental birds the aorta and the brachiocephalic, coronary and splenic arteries were thus affected, while in the control chicks, on an identical diet except for cholesterol and cottonseed oil, no atheromatous deposits developed.

Furthermore, it has been possible to produce experimentally the same type of vascular lesion as occurs spontaneously in the species under consideration. The changes induced in the chick arteries resemble in their appearance the earliest spontaneous lesions described in the literature.[4a] Their location corresponds to that of the usual avian spontaneous atherosclerosis,[4a] although no abdominal aortic lesions have been seen.

The changes which were produced with the short feeding period not only reproduced the spontaneous chick lesions but also simulated the earliest "fatty streaks" of man, such as are seen in human vessels from the age of 6 months onward and are generally regarded as reversible.[11] These fatty lesions in man are often found just above the sinuses of Valsalva in the ascending aorta, as well as in the sites of the most marked human arteriosclerosis, the descending and the abdominal aorta. The localization of these early human lesions is therefore more like that of the experimental lesions than is the site of the more advanced arteriosclerosis. The difference in localization of the advanced spontaneous avian and human lesions is probably associated with anatomic and functional differences between the chick and man. In the chick the

10. Hurwitz, M., and Friedberg, L.: Arch. Path., to be published. Katz, L. N.; Saunders, A.; Megibow, R. S., and Carlen, S.: Am. J. M. Sc. **200**:731, 1940.

11. (*a*) Leary, T.: Arch. Path. **32**:507, 1941. (*b*) Ophüls, W., in Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933, chap. 9, p. 249.

brachiocephalic arteries arise from a large ampulla just above the left ventricle; hence it would be expected that the greatest traction and torsion would be exerted here rather than at the aortic arch, which is the site of branching in man.

While definite intimal plaques were produced in our chicks, it should be noted that no secondary changes, such as fibrosis, hyalinization or degeneration with ulceration and thrombosis, resulted. It is possible that such lesions might be produced with more prolonged cholesterol feeding, especially if this were followed by an interval of cholesterol withdrawal, as has been done in experiments with the rabbit.[12] However, birds rarely if ever show in spontaneous lesions the deformed ulcerated surfaces with thrombosis seen in advanced human lesions, although well marked atherosclerosis with calcification does develop. Our results suggest that the lipoid material appears first in the intima, but they do not reveal whether this comes about by direct diffusion from the blood or by invasion of the intima by lipoid-bearing histiocytes.

From our results it is evident that atheromatosis is produced equally readily in the chick and the rabbit. It takes thirty to forty-five days to produce microscopic atheroma and fifty-five to seventy-eight days to produce gross lesions in the rabbit.[13] In our chicks the intimal changes were seen microscopically after forty-two days of cholesterol feeding and grossly after forty-nine days. This is comparable with the latent period in the rabbit. The rabbit must ingest 11 to 18 Gm. of cholesterol before microscopic atheroma is seen and 30 Gm. before gross changes are evident.[13] In the chick, we estimated that 48 Gm. of cholesterol was ingested before microscopic atheroma was seen and 55 Gm. before the first gross lesions appeared. In gross and microscopic appearance the lesions in the chick are similar to the early lesions in the rabbit, although none of the fibrosis and hyalinization which the rabbit shows was found in the chicks. Further, the lesions in the two animals are similar from the standpoint of localization. In both, the ascending aorta and the aortic arch, the coronary arteries and the splenic arteries are involved early. However, in the rabbit there characteristically develops atheroma of the pulmonary arteries, of the cardiac valves and of the large veins, which thus far we have not seen in the chick. While these results may remove some of the objections previously raised to the use of rabbits for studies of arteriosclerosis, no claim is made that the mode of production of the lesions in the rabbit and the chick, by exogenous cholesterol, bears any but an indirect relation to the mode of genesis of the lesions in man.

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12. Anitschkow, N.: Verhandl. d. deutsch. path. Gesellsch. **23**:473, 1928. Leary.[11a]
13. Anitschkow, N.: Ergebn. d. inn. Med. u. Kinderh. **28**:1, 1925.

In the past the difference in results of cholesterol feeding between the rabbit and other animals was attributed to the herbivorous diet of the former, in which cholesterol is lacking and only phytosterol is present. Some other explanation must be sought for the accumulation of cholesterol in the chick tissues, since this sterol is normally present in the chick diet. Why the rabbit, the guinea pig and the chick show enormous elevations of blood cholesterol and infiltration of numerous organs on cholesterol-rich diets while the rat shows little or no elevation of blood cholesterol and accumulates cholesterol only in the liver [14] cannot be stated at the present time. Nor are there any data enabling us to compare man with either of these groups of animals. It is known that all the species under consideration absorb and synthesize cholesterol and that likewise they both excrete and destroy it. Further it is known that external dietary factors, such as lipocaic and choline, may play a role in the deposition of cholesterol. The amount of fat needed for effective absorption of cholesterol varies in the different animals.[15] However, it must be admitted that at the present time knowledge is insufficient to permit any conclusions as to the comparative physiology of cholesterol. The establishment of the significance of cholesterol atheromatosis in an omniverous animal in relation to human arteriosclerosis hinges on a clarification of the entire subject of cholesterol metabolism.

The relation of sex to the incidence of spontaneous arteriosclerosis in man and in birds is of great interest. In both species there is a greater incidence of spontaneous vascular lesions in males than in females. Feeding experiments in the animals show a clearcut sex difference in the ability to tolerate cholesterol. For example among rats the livers of males enlarge more than do those of females on cholesterol feeding [16] and show a higher concentration and a higher absolute amount of cholesterol.[17] This sex difference was exaggerated in undernourished animals despite their sexual infantilism,[17] which may be related to the marked cholesterol effects in undernourished cockerels. It has been suggested that females have a more efficient mechanism for disposing of cholesterol than have males.

A number of points remain to be further explored. Our series of heart weights in relation to body weights suggests that in chicks as in rabbits cholesterol feeding is associated with cardiac hypertrophy.[10]

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14. Cook, R. P., and McCullagh, G. P.: Quart. J. Exper. Physiol. **29**:283, 1939.

15. Cook, R. P.: Biochem. J. **30**:1630, 1936; **31**:410, 1937; **32**:1191, 1938.

16. Okey, R.: Proc. Soc. Exper. Biol. & Med. **30**:1003, 1933; J. Biol. Chem. **107**:207, 1934.

17. Gillum, H. L., and Okey, R.: J. Nutrition **11**:309, 1936.

Further observations of heart weight in the presence of undernutrition and underdevelopment and a larger series of birds are necessary before definite conclusions can be drawn.

The effect of feeding vegetable oil alone, without cholesterol, has been ruled out as a source of arterial lesions in the rabbit.[8] This remains to be done for the chick.

SUMMARY AND CONCLUSIONS

Twenty-four 10 day old cockerels were divided into two equal groups. One group was fed an adequate diet for fifteen weeks. The second group was placed on the same diet plus 2.5 to 5 per cent cholesterol in cottonseed oil. In none of the control cockerels did vascular lesions develop. In 7 of the 12 cholesterol-fed birds (the remaining 5 dying within eight weeks) atheromatous deposits developed in the aorta. Of these 7 chicks, 3 had intimal atheromatous lesions in the coronary arteries with resultant narrowing of the vessel lumens; 2 of the 7 showed similar changes in the splenic arteries. The hearts of the cholesterol-fed birds weighed slightly more in proportion to the body weights than did those of the control series.

The chick is an omnivorous animal in which experimental atheromatosis can be readily produced by feeding a high cholesterol diet. The induced arterial lesions resemble the earliest spontaneous atheromatosis which develops with advanced age in chickens.

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LOCALIZED PLEURAL MESOTHELIOMA

INVESTIGATION OF ITS CHARACTERISTICS AND HISTOGENESIS BY THE METHOD OF TISSUE CULTURE

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NEW YORK

The study of tumors by the method of tissue culture furnishes valuable information which serves to augment the knowledge which may be gained from purely morphologic studies. It has a special value in regard to tumor types about the histogenesis and classification of which there is controversy. In the present communication we wish to report our study of a primary localized pleural tumor and our deductions regarding the nature of the cells composing it. Before doing so it seems necessary to review the speculations already made regarding all pleural tumors, based on morphologic, embryologic and experimental studies, because the conclusions reached are both confused and conflicting.

During the past seventy years well over two hundred papers have been written reporting tumors of reputed pleural, pericardial and peritoneal origin. By far the largest number of these neoplasms have been found in connection with the pleura. These have been divided into two main groups of supposed primary neoplasms: the diffuse and the localized. The diffuse form usually thickens the pleura, invades the underlying tissues, such as the lung, diaphragm, pericardium and thoracic wall, to a limited extent and either does not metastasize at all or is found only in the mediastinal nodes. Occasionally the tumor invades the peritoneum as well, extending along its surface without deep penetration. The histologic features are striking because there are two widely different types of tissue found in the same tumor. One of these is distinctly of an epithelial appearance and consists of solid cords or hollow tubes of cells, which sometimes seem to secrete a mucinous material. The other tissue assumes the appearance of a fibrosarcoma: spindle cells arranged in bundles, often with reticulin or collagen fibers between them. The epithelioid elements sometimes have suggested the hyperplastic prolifera-

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tion of vascular endothelium when the cells assume a rounded or polygonal form, but more often seem truly epithelial with cuboidal or cylindric cells arranged about lumens. The mixed character of these tumors has not by any means been noted in all reported cases. Often the growth has epithelioid elements and only an ordinary fibrous stroma, and rarely the growth appears to lack all epithelioid elements. The localized growths, on the other hand, do not have the epithelioid elements but seem more like fibroma or fibrosarcoma. They may grow to a very large size, but only rarely do they metastasize. A majority of the tumors are circumscribed, but some infiltrate surrounding tissues.

Speculations regarding the cellular origin of these tumors have been along four principal lines: 1. They are all secondary to occult primary cancers elsewhere, and there is no such thing as a primary tumor of pleural lining cells. In spite of all that has been written, this is still the recently expressed opinion of some serious students of the subject, such as Robertson,[1] W. Fischer [2] and Willis [3] They point out that it is easy at autopsy to overlook a tiny primary carcinoma in a bronchus, and it is well known that such a tumor may give rise to extensive metastases without itself ever growing to an appreciable size. Unquestionably some of the reports have dealt with secondary cancers which have displayed a tendency to spread through the pleural, pericardial and peritoneal tissues in this peculiar fashion, but it does not seem possible to explain all of the tumors on this basis.

2. Some of the older writers wished to describe these tumors as having their origin from the endothelium of lymphatic vessels. This hypothesis was based on the belief that lymphatic-vascular endothelium could produce epithelium-like structures. During the past twenty-five years, a period during which the types of tumorous growths from vascular endothelium have become more familiar, the conception has had little support. Among the older adherents of this idea were Schulz,[4] Böhme [5] and Herxheimer and Reinke,[6] and recent adherents are Grossek [7] and Froriep.[8]

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1. Robertson, H. E.: J. Cancer Research **8**:317, 1924.

2. Fischer, W., in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1931, vol. 3, pt. 3, pp. 558-570.

3. Willis, R. A.: J. Path. & Bact. **47**:35, 1938.

4. Schulz, R.: Arch. f. Heilk. **17**:1, 1876.

5. Böhme, M.: Virchows Arch. f. path. Anat. **81**:181, 1880.

6. Herxheimer, G., and Reinke, F.: Ergebn. d. allg. Path. u. path. Anat. **16**: 225, 1914.

7. Grossek, R.: Ztschr. f. Krebsforsch. **35**:435, 1932.

8. Froriep, E.: Folia hæmat. **51**:452, 1934.

3. Fischer-Wasels,[9] Marras [10] and Scheidegger [11] have favored an origin from remnants of pulmonary epithelial tissue reputed to have been left in the pleura during embryonic life or to have become pinched off by the process of scarring. This conception is purely hypothetic.

4. Finally, there is the most favored hypothesis, which ascribes the origin of most of the diffuse tumors to pleural mesothelial cells. This has been accepted by Krumbein,[12] Zeckwer,[13] Kux,[14] Klemperer and Rabin [15] and Cornil and co-workers.[16]

This theory proposes to explain the production of both carcinomatous and sarcomatous elements either separately or in the same tumor from pleural mesothelial cells on the basis of the embryologic work of the Hertwigs [17] and the experimental work of Maximow [18] and Young.[19] The Hertwigs [17] showed that the lining of the pleuropericardial cavity apparently develops from celomic epithelium which is split off from the mesoderm. Celomic epithelium gives rise to definite glandular structures as well as to serosal covering cells. Maximow [18] showed by means of tissue culture that the mesothelium or covering cells of the serosal surfaces can form cells of a fibroblastic appearance, together with collagen fibers. It has also been shown by Young [19] that the pleural serosal cells in a rabbit will undergo extensive hyperplastic and metaplastic proliferative changes, forming squamous epithelia as well as glandlike spaces lined by swollen epithelial-like cells, if subjected to long-continued irritation by a mixture of sudan III and sodium cholate in olive oil. Therefore, if these findings are correct, there is no difficulty in supposing that these potentially versatile serosal covering cells of the pleura, pericardium and peritoneum may produce epithelial tumors, fibroblastic tumors or tumors in which both fibroblastic and epithelial elements are combined.

There is a further analogy which may be used in support of the hypothesis that cells lining a body cavity have the ability to form tumors composed of both fibrous and epithelial elements. The malignant tumors which develop from the synovial cells of joints, tendon sheaths and

9. Fischer-Wasels, B.: Ztschr. f. Krebsforsch. **37**:21, 1932.
10. Marras, S.: Pathologica **26**:8, 1934.
11. Scheidegger, S.: Ztschr. f. Krebsforsch. **42**:93, 1935.
12. Krumbein, C.: Virchows Arch. f. path. Anat. **249**:400, 1924.
13. Zeckwer, I.: Arch. Int. Med. **34**:191, 1924.
14. Kux, E.: Virchows Arch. f. path. Anat. **272**:650, 1929.
15. Klemperer, P., and Rabin, C. B.: Arch. Path. **11**:385, 1931.
16. Cornil, L.; Audibert, V.; Montel, L., and Mosinger, M.: Bull. Assoc. franç. p. l'étude du cancer **27**:51, 1938.
17. Hertwig, O., and Hertwig, R.: Ztschr. f. Naturwissensch. **8**:1-150, 1881.
18. Maximow, A.: Arch. f. exper. Zellforsch. **4**:1, 1927.
19. Young, J. S.: J. Path. & Bact. **31**:265, 1928.

bursas are composed of fibrosarcomatous tissue with spindle-shaped cells and argyrophil fibers between the cells, mixed with glandlike slits or spaces lined by cells of a distinctly epithelial aspect which, in some instances, secrete mucoid material. The only difference between the synovioma and the complex pleuroma (as Cornil and associates [16] elect to call it) is that the synovioma is a localized tumor, while the complex pleuroma is spread out over a broad surface.

The localized pleural tumors, since they do not have glandlike structures in them but are composed of tissues which are usually of a connective or vascular type, are not ascribed to the mesothelial covering cells by most authors. Klemperer and Rabin [15] spoke for the great majority when they insisted that these localized growths come from subpleural areolar tissues. But recently Cornil and associates [16] expressed doubt about this. They pointed out that the diffuse "complex pleuromas" can form fibroma-like and sarcoma-like tissue and hence it is not impossible to suppose that the localized "connective pleuromas" may come from the pleural mesothelium.

When one consults the reports of these localized tumors, the information furnished is exceedingly meager. Hardly any have been studied with special stains, and few of the reports have proper illustrations. One gains the impression that the large majority of the neoplasms are composed of spindle cells and have the appearance of fibroma or more commonly of fibrosarcoma. The intercellular fibers vary greatly, sometimes in the same tumor (Dorendorf [20]). Myxomatous areas occur in some (Mehrdorf [21]; Andrus [22]; Klemperer and Rabin [15]). Henke's [23] and Fischer's [2] tumors were rich in blood vessels. Some have a suggestion of epithelial or endothelial-like cells (Heuer [24]; Sala [25]). Rounded cells appearing with the spindle cells have been described (Jacobi and Bolker [26]). No localized tumors have been described which had an admixture of unmistakable epithelial glands, such as are so commonly found in the diffuse tumors. On the other hand, it is only proper to recall that some of the diffuse tumors seem purely fibrosarcomatous (Barret and Elkington [27]; MacMahon and Mallory [28]).

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20. Dorendorf, H.: Deutsche med. Wchnschr. **40**:225, 1914.

21. Mehrdorf, R.: Virchows Arch. f. path. Anat. **193**:92, 1908.

22. Andrus, W. D.: J. Thoracic Surg. **4**:236, 1935.

23. Henke, F.: Mikroskopische Geschwulstdiagnostik, Jena, Gustav Fischer, 1906, p. 238.

24. Heuer, R.: Ann. Surg. **79**:670, 1924.

25. Sala, A. M.: Arch. Path. **9**:950, 1930.

26. Jacobi, M., and Bolker, H.: Arch. Path. **13**:534, 1932.

27. Barrett, N. R., and Elkington, J. S.: Brit. J. Surg. **26**:314, 1938.

28. MacMahon, H. E., and Mallory, G. K.: Am. J. Path. **4**:387, 1928.

REPORT OF A CASE

The patient was a woman aged 43 years. She had had a cough for about two years. She had been given roentgen therapy elsewhere near the beginning of this period, without improvement, and it was supposed that she had an aneurysm of the first part of the descending aorta. An exploratory operation proved that this was not so. Finally, Oct. 11, 1939, a total left pneumonectomy was performed. The tumor measured 10.5 by 8 by 7 cm. It was adherent to the pleura of the lower lobe of the left lung and lay in that tissue, making a projection in the bronchus and extending outside along the tissues at the hilus. In the inferior part of the same lobe was a separate nodule of tumor, 35 mm. in diameter. The main tumor was circumscribed but not encapsulated, and its cut surface was of relatively uniform fleshy consistency, reddish gray occasionally tinged with yellow, nodular and slightly bulging. At its mediastinal projection it had been cut through. The postoperative course was stormy and included several operations for empyema, but the patient finally recovered except for an unhealed fistula. In February 1941 the discharge from this became profuse, and it was thought that the fistula was due to persisting tumor growth. Roentgen therapy was given from March 7, 1941 through May 16, 1941 through three 15 by 15 cm. fields, totaling 5,000 r in 50 to 100 r doses. The factors were: 200 kilovolts, 25 milliamperes; target-skin distance, 80 cm.; filter, 0.5 mm. copper plus 1 mm. aluminum. The patient was temporarily improved, but there was a massive local recurrence, and she died Dec. 12, 1941, twenty-six months after operation. At autopsy tumor tissue was found in the space formerly occupied by the left lung and pleura, around the stump of the left main bronchus and in the adjacent mediastinum. There was a small metastasis in the head of the pancreas and another in the right ovary. The uterus contained several small tumors, diagnosed as leiomyomas.

Microscopic Study.—The microscopic appearance of the tumor at operation was basically the same everywhere, although there were quantitative variations in the content in different parts (fig. 1). The characteristic tumor cell was medium sized and fusiform, with a relatively large and prominent hyperchromatic nucleus, correspondingly shaped. Nucleoli were small and inconspicuous. Mitoses were regular and averaged one in every two or three high power fields. The cytoplasm was finely granular, dense and faintly acidophil. The cells were closely packed and tended to be arranged in interlacing bundles. In the primary tumor many of the cells had long slender reticulin fibers between them and occasionally even collagen fibers. The content of connective tissue fibers varied greatly in different areas. It was minimal in the metastasis, where there were almost no fibers. In all parts there were numerous vascular slits and tubes. Most of these had empty lumens, although a few contained scattered erythrocytes and rarely white blood cells. These vessels when they were large had a definite endothelial lining. As they became smaller, however, this occasionally became less well defined, and some of the smallest seemed to be simply unlined slits in the tumor cells. Where endothelium was present it usually rested on a very thin reticulin sheath, but where it was absent there was no reticulin sheath. The endothelium was always a characteristic flattened pavement; the cells never assumed an epithelial aspect. At its periphery the tumor tended to be confined within a fibrous sheath, but this was not a true capsule, for tumor had invaded it in places.

Tissue Culture.—Portions of the primary tumor and of the metastasis in the lung were cultivated by the Maximow double cover slip method for eighty-three days. In the beginning a mixture of human and rat plasma, human placental serum

and extract of adult rat spleen was used as a growth medium. Later on, the mammalian plasmas were replaced with chicken plasma.

(*a*) Wandering cells: Within twenty-four hours after explantation some of the cultures showed outgrowths of fixed cells. This development was preceded by the appearance of large numbers of phagocytic wandering cells, which were indistinguishable from macrophages in form, behavior and staining reactions (fig. 2*B*). When neutral red was applied supravitally to the cultures, it colored a few

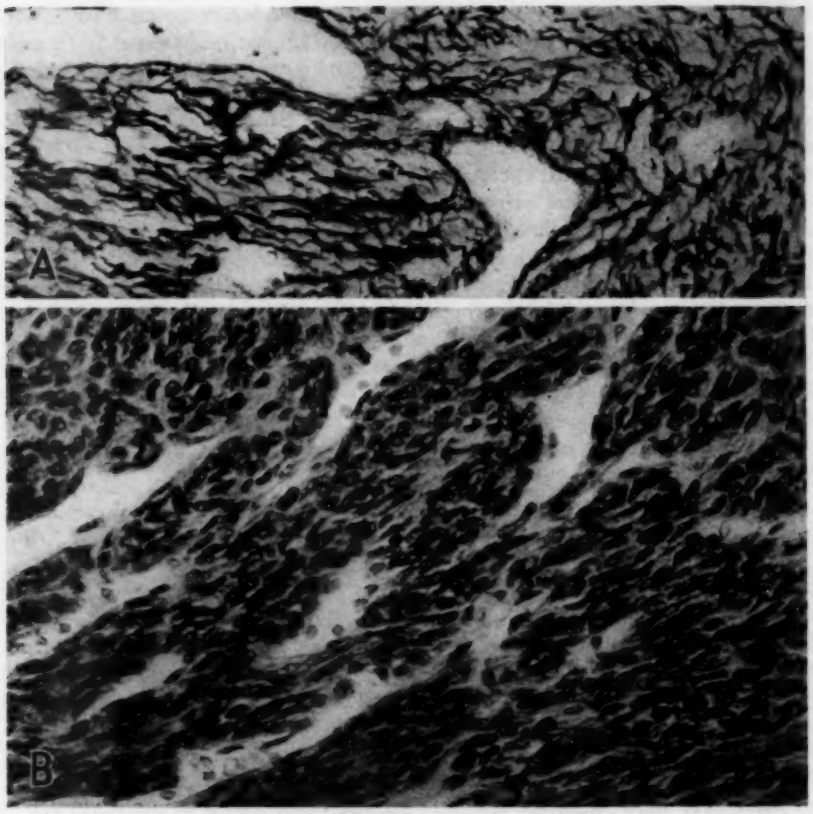

Fig. 1.—Histologic characteristics of the pleural tumor. Note the arrangement of spindle cells and blood vessels in *B* and of reticulin fibers in *A*.

small granules in the fixed cells pale pink, but quickly stained brilliant scarlet large numbers of granules, varying in size, in the wandering cells. One fourth of the cultures after forty-five days in vitro were treated with a dilute solution of trypan blue in Locke solution, which was allowed to remain in contact with them for two days. At the end of this period a few purplish vacuoles could be seen by transmitted light in both fixed and wandering cells. At the end of seven days, however, the fixed cells were colorless, and large accumulations of dye were to be seen in the wandering cells. Inspection of the explants, in which the wandering cells stood out boldly by reason of their coloration, showed that they did not

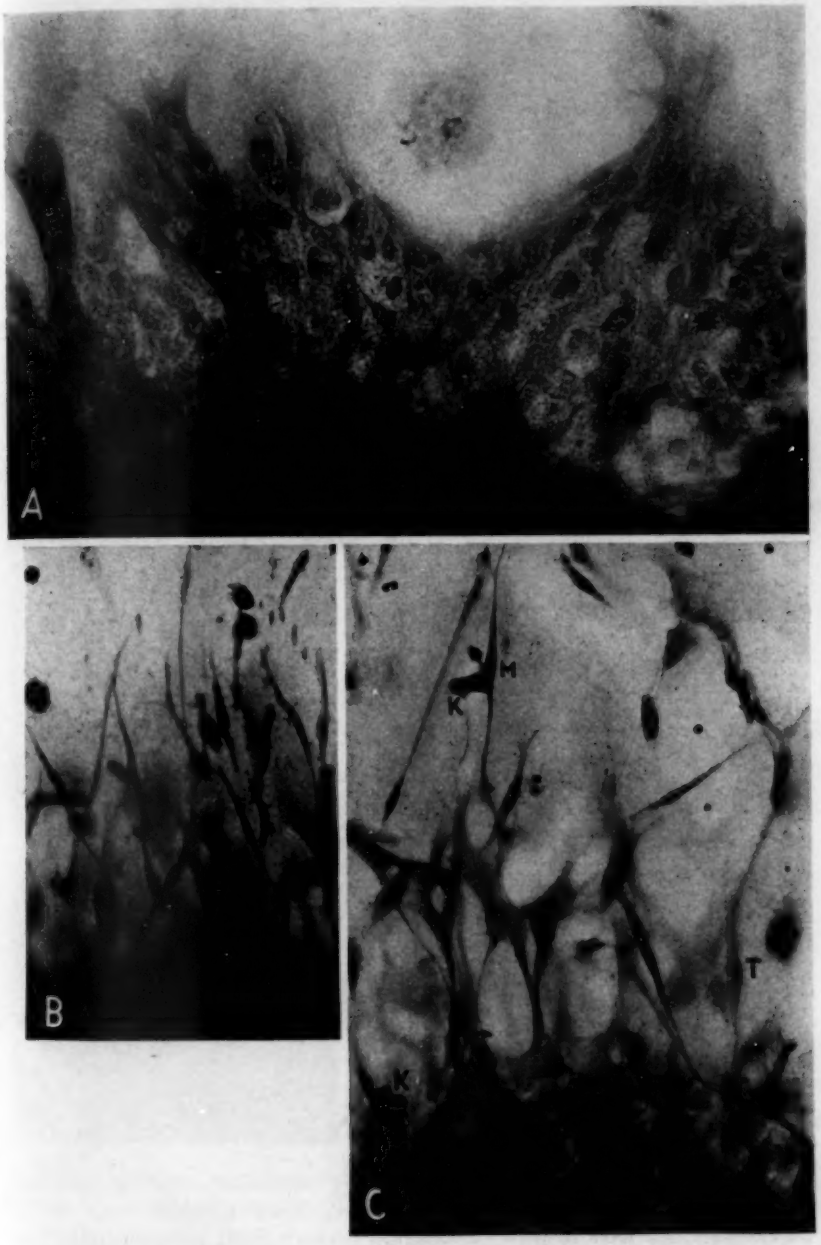

Fig. 2.—*A*, tissue culture from a pulmonary metastasis of the pleural tumor. One week in vitro; fixation in Zenker's fluid; phosphotungstic acid–hematoxylin. *B*, tissue culture from the same material as *A*. One week in vitro; Masson's trichrome stain; same magnification. *C*, another portion of the same culture showing shelflike growth. *K* indicates karyokinesis; *M*, macrophage; *T*, tumor cell.

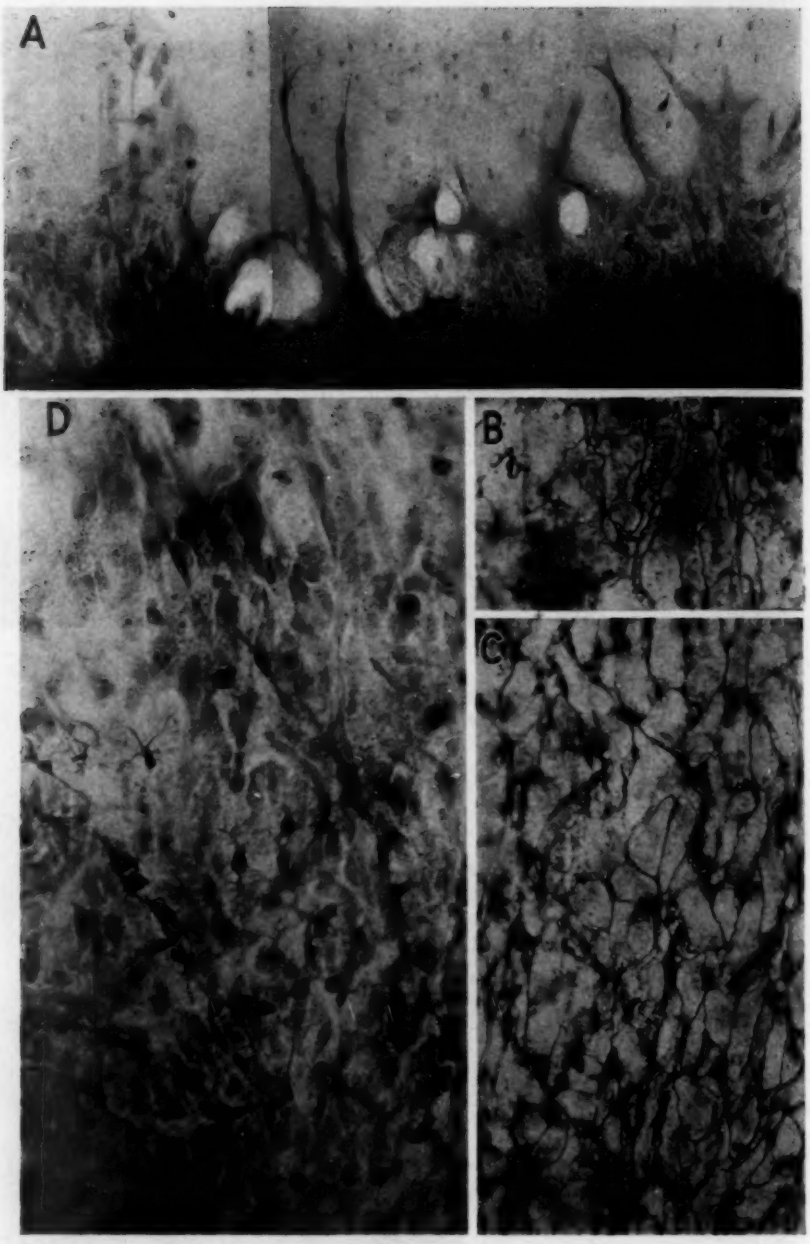

Fig. 3.—*A*, tissue culture from pulmonary metastasis, showing membrane formations, isolated cells, solid cell bands and typical epithelial holes without fibrinolysis. One week in vitro. Fixation in Zenker's fluid; phosphotungstic acid-hematoxylin. *B*, tissue culture from the main tumor, showing cell borders blackened by silver nitrate. One month in vitro. *C*, tissue culture of pleura from lung of a 3 week old rat. Five days in vitro; silver nitrate stain. *D*, outgrowth from pleura of a 3 week old rat. One week in vitro; fixation in Helly's fluid; Masson's trichrome stain; same magnification as *C*.

make up the bulk of the tissue in any area, but were scattered rather thickly throughout. They were always very much more numerous in cultures of the primary tumor than in those from the metastatic nodule. These cells were seen to divide by mitosis (fig. 2 *C*). They still persisted in fairly large numbers at the time cultivation was discontinued. They were never observed to develop from fixed cells in the outgrowth. There seems to be no great difficulty in accounting for the presence of large numbers of these cells in the tumor, since the serous membranes normally contain many macrophages and undifferentiated mesenchymal cells,

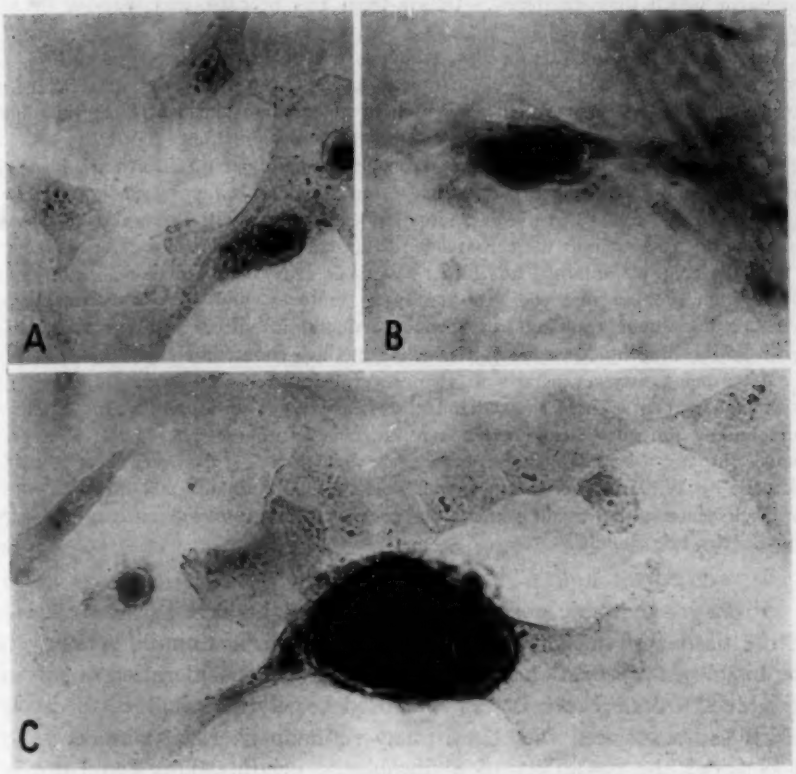

Fig. 4.—*A, B* and *C*, three stages in the formation of a tumor nodule in vitro. This nodule is taken from a one week culture containing many nodules in different stages. Fixation in Helly's fluid; phosphototungstic acid–hematoxylin.

the mediastinum is a copious source of macrophages, and the lung is a similar source of alveolar phagocytes. Their persistence in the cultures may be of some interest.

(*b*) Fixed cells: The fixed growth in these cultures took the form of cords and membranes composed of moderately broad flattened cells approaching the squamous type, but often bearing short, sharp pseudopodia when at the edge of an outgrowth or when isolated (figs. 2 *A* and 3 *A*). These membranes, which were generally of the pavement type, tended to grow several layers thick, thus leading

to the formation of a shelving, terraced outgrowth at the margin of the explant (figs. 2 *C* and 3 *A*). When treated with silver nitrate in the manner of stretch preparations of the serous membranes, the outgrowths appeared as mosaics of contiguous cells, marked off one from another by rather irregular black borders (fig. 3 *C*). These borders could also be seen throughout the explant. The cells surrounded by them appeared to form the bulk of the tissue. Wandering cells did not exhibit these borders.

Areas of spontaneous necrosis occurred in the explant during the course of cultivation, their occurrence bearing little or no relation to the thickness of the tissue in which they developed. Debris from this necrosis was ingested by the wandering cells.

Liquefaction of the plasma clot, which is a troublesome factor in cultures of normal epithelium and of carcinoma, was absent from the areas of fixed cells, even when they were grown in a medium of mammalian plasma. Large aggregations of wandering cells sometimes produced fibrinolysis.

Cells of the membranous type had a tendency to wander off from the advancing membrane and form new tumor nodules some distance away by undergoing frequent and rapid mitosis in the new situation (fig. 4). It will be seen that in some portions of these nodules the cells tended to assume a spindle shape (fig. 4 *B*).

At the time we obtained this tumor (October 1939) we were not able to apply sliver impregnation methods successfully to cultures, and so we do not know whether the tumor continued to produce reticulin in vitro. No fibers stainable with aniline blue by Masson's [29] trichrome method were found.

After being fifty-seven days in vitro several cultures were stained supravitally with janus green. These showed mitochondria as aggregations of round granules surrounding the nucleus and extending irregularly into the cytoplasm. An occasional short rod-shaped granule was seen, but none of the very long threads pictured by W. H. Lewis in embryonic chicken endothelium [30] and mesothelium.[31] A large centrosphere could sometimes be seen adjacent to the nucleus and surrounded by mitochondria.

COMMENT

From its anatomic position, this tumor might have arisen from any of the tissues of the lung, the pleura or the mediastinum. A study of the histologic preparations made from it led to a consideration of several possible sources. Since it was composed largely of spindle-shaped cells which had associated with them many reticulin fibers, it was necessary to consider the possibility of a fibroblastic origin. This could not be excluded, although in its marked vascularity and general appearance the tumor did not coincide with tumors that had been proved to be fibroblastic. The large number of blood vessels forced consideration of an origin from vascular endothelial cells, from pericytes or from other cellular components of blood vessels. We knew of no neoplasms of any of these cells which assumed this aspect and therefore rejected each of these components as a probable origin. Bronchial epithelium, although capable of the fromation of acini, squamous epithelial cells and oat-shaped

29. Masson, P.: J. Tech. Methods **12**: 75, 1929.
30. Lewis, W. H.: Am. J. Anat. **30**:39, 1922.
31. Lewis, W. H.: J. Exper. Med. **38**:257, 1923.

cells does not, so far as we are aware, produce large spindle-shaped cells and reticulin fibers, and we therefore rejected these cells as a probable source. If alveolar lining cells are derivatives of the reticulo-endothelial system, they might be considered as a possible source for this tumor, since the suggestion has been made that these cells may be transformed into fibroblasts. Since this hypothesis rests on two unproved premises, it was not given very serious consideration, although not abandoned. Finally we considered the possibility of an origin from pleural mesothelium since it is known that this tissue can form tumors with spindle cells and reticulin fibers as an important component and that pleural mesothelium in vitro can form spindle cells and connective tissue fibers.

It is apparent that histologic examination of this tumor did not permit us to make an exact diagnosis. We therefore turned to the information furnished by the tissue cultures for further enlightenment.

Since no fibroblastic outgrowth was ever obtained in the tissue cultures, we felt justified in excluding the possibility of a fibroblastic origin for the tumor.

Pericytes are easily distinguishable in vitro on a morphologic basis. They never form a membrane of any sort but behave as discrete elements entirely (Murray and Stout [32]). They can therefore be readily rejected as a source for the tumor.

No tumors of known vascular origin which we have studied in vitro have shown the type of growth appearing in the cultures from this tumor. They have produced solid and hollow capillary formations, and sheets of fibroblast-like cells in which black borders could be demonstrated by silver nitrate staining, but never pavement membranes such as these. Studies of normal vascular endothelium in this laboratory and others (e. g., Maximow,[33] W. H. Lewis [34] and Schopper [35]) show that it grows in vitro as solid or hollow capillary sprouts and plexuses, as mesenchyme or as spindle-shaped connective tissue cells between which reticulin fibers may be found, and cell borders blackened by silver nitrate. There is no record of its producing membranous outgrowths such as characterize this tumor.

In special situations, however, endothelium may behave differently. Endocardium from the ventricular trabeculae grows only in a form that can hardly be distinguished from fibroblasts (Nishibe [36]). But endothelium from the sinusoids of the liver may sometimes form membranes, though this is rare and the membranes are distinguishable from meso-

32. Murray, M. R., and Stout, A. P.: Am. J. Path. **18**:183, 1942.
33. Maximow, A.: Klin. Wchnschr. **4**:1486, 1925.
34. Lewis, W. H.: Bull. Johns Hopkins Hosp. **48**:242, 1931.
35. Schopper, W.: Beitr. z. path. Anat. u. z. allg. Path. **88**:451, 1932.
36. Nishibe, M.: Arch. f. exper. Zellforsch. **8**:367, 1929.

thelial outgrowths (Lewis [30]). According to Matsui,[37] endothelium from Descemet's membrane characteristically takes on an epithelial arrangement in vitro, although the fibroblastic form of growth is also encountered. If alveolar epithelium is accepted as a reticuloendothelial derivative, it is conceivable that it should behave in vitro like endothelial cells from the sinusoids of the liver or from the endothelial layer of the cornea. An explanation of our tumor growth on this basis, however, would rest on one unproved hypothesis and one pure assumption.

From the morphologic aspects of the tissue cultures alone, it is impossible to exclude bronchial endoderm as a source for this tumor. The membranous growth, shelf formation, spontaneous necrosis and development of tumor nodules de novo in vitro are all consistent with carcinoma. But the absence of clot liquefaction would represent a physiologic anomaly for human carcinoma cultures grown in mammalian plasma.

We have been unable to find any account of neoplasms of serosa studied in vitro. But students of normal mesothelium in tissue culture agree in describing outgrowths strikingly similar to those from this tumor. Without exception, they state that the growth pattern is typically epithelial. Polygonal cells with large oval clear nuclei, and a distinct thin ectoplasm spread membrane-like against the cover slip or over the surface of the clot. Holes are often formed in the outgrowth by the fibrinolytic action of the cells on the clot. Treated with silver nitrate, the membrane appears as a mosaic of cells with black borders.

W. H. Lewis,[31] studying the embryonic chicken heart, observed characteristic mesothelial membranes derived from mesenchyme. He expressed the belief that "mesothelium is in the nature of a transformation in the form of the cells of the primitive mesoderm, due to environment," and he observed all stages of transformation from the bipolar and multipolar reticular cells of mesenchyme to the flat mesothelial forms.

Maximow,[18] cultivating omentum and mesentery of adult rabbits in vitro, obtained mesothelial growths quite distinct from the outgrowths produced by vascular endothelium, which latter never formed epithelial membranes. The mesothelial cells sometimes formed layers of high cuboidal elements and gave rise to compact epithelial-like islands. Necrosis as a result of mechanical injury was common among mesothelial cells. Under conditions of inflammation mesothelium developed into a typical fibroblastic reticulum.

Schopper,[35] studying omentum and pleura from young guinea pigs, did not observe formation of connective tissue in vitro by normal mesothelial cells. But following intraperitoneal injections of kieselguhr (essentially the same as purified silicious earth U. S. P.) these cells

37. Matsui, J.: Arch. f. exper. Zellforsch. **8**:533, 1929.

took on the form and characteristics of typical fibroblasts. In their growth habits they were clearly distinguishable from vascular endothelium.

Chlopin,[38] using rabbits of various ages, described the characteristics in vitro of normal serous membranes from seven different regions. At the periphery of the outgrowing membranes, single cells often wandered off alone, and solid bands of cells projected into the medium. He regarded mesothelium as a histologically determined tissue which can be qualitatively distinguished from epithelial tissues and from mesenchymal derivatives, and has no capacity to develop into the latter. In vitro the follicular elements of the gonads produce growths similar to mesothelial outgrowths; these two tissues are included by him in a special "zölodermal" tissue group.

These four authors' figures of mesothelium in vitro, as well as our own cultures of normal pleura, pericardium and mediastinum from young rats (fig. 3 *B* and *D*), can hardly be distinguished from cultures of the tumor. Our cultures of normal serosa showed no liquefaction, but we used mixed plasmas in the medium, instead of the homogenous plasma used by Maximow, Schopper and Chlopin or the entirely liquid medium of Lewis. If the discrepancy in the data on fibrinolysis is explained by this difference in the constitution of the mediums, all the available tissue culture studies of mesothelium seem consistent with the appearance and behavior of this tumor in vitro and provide no evidence which would disqualify it from being a mesothelioma.[39]

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38. Chlopin, N. G.: Arch. f. exper. Zellforsch. **19**:86, 1937.

39. It is only proper to record that Dr. A. M. Pappenheimer, who studied this case at autopsy, does not agree with our interpretation. He has made the following note on it, which we quote verbatim with his permission. " . . . The tumor is an unusually interesting one, and there has been a difference of opinion between the surgical pathologists and the staff of the department of pathology of Columbia University as to the correct diagnosis. A preliminary diagnosis of fibrosarcoma by Dr. Stout, made from the biopsy specimen, was later amended to a diagnosis of pleural mesothelioma, on the basis of tissue cultures studied by Dr. Margaret R. Murray. In these, the explanted cells grew out in coherent sheets, and the resemblance of these cells to those obtained from explanted rat mesothelium was considered by Dr. Murray as decisive evidence of their derivation.

"We regret that we cannot accept this interpretation. The tumor does not resemble the two specimens in our files which are labeled pleural mesothelioma; these have more or less of an alveolar structure and are composed of rounded or polygonal epithelial-like cells, conforming to other tumors described under this title in the literature (see Geschickter, Ewing, Stout and others). The slow development of the growths, extending over a period of several years, seems to us also to argue against this diagnosis, since the tumors diagnosed as mesothelioma are in general rapidly growing and highly malignant.

(Footnote continued on next page)

SUMMARY

When the evidence from the sections is combined with the evidence from the tissue cultures, practically every possibility except mesothelioma seems to be excluded on one ground or another. Therefore, notwithstanding the conflicts of evidence on some points relating to the potencies of serosal mesothelium, it seems highly probable that the tumor described here represents a primary neoplasm of the pleural mesothelium.

"Furthermore, we are not prepared to accept the thesis that the diagnosis can be established solely on the appearance of the cells in tissue culture, particularly when there is such divergence from their appearance in the parent tumor. Indeed, one cannot be sure that the growing cells are derived from the neoplastic elements, and not from other components—endothelial or mesothelial—which may have been included in the piece taken for examination.

"Our own diagnosis leans to leiomyosarcoma, probably metastasizing from one of the uterine tumors diagnosed as myoma. Reexamination of the uterus shows areas of grayish white tissue, identical in gross appearance with the thoracic tumor, composed microscopically of diffusely growing, very cellular myomatous tissue. In some places, these cells surround glandular, epithelial-lined spaces—adenomyomatous areas. The ovarian stroma, particularly that of the right ovary, has been transformed into similar tissue.

" . . . (The uterine and mediastinal tumors show) close resemblance in the arrangement of the interlacing whorls and strands of cells, in the shape and size of the nuclei and in the paucity of mitotic figures. Unfortunately, we have not been able to demonstrate myoglia fibers either in the uterine or in the mediastinal tumors. Malignant metastasing uterine myoma showing very little deviation from the benign type has been repeatedly reported, although it is less common than the frankly sarcomatous type. The protracted course of the disease in this patient is in accord with this diagnosis. An almost identical case is that reported by Paul Steiner in the *American Journal of Pathology* (**15**:89, 1939). In this paper will be found a review of similar cases in the literature."

Because of this criticism, which was made after this paper was accepted for publication, we withdrew the paper for reevaluation. After due consideration, we have determined to adhere to our original interpretation, fortified by our belief that the cells which grew from the explant could not have come from the pleura in one instance at least, because the specimen in that instance was taken from a separate nodule within the lung substance, and that it is extremely unlikely that they came from vascular endothelium because no normal vascular endothelium ever grew in this fashion. The growth in vitro certainly came from the tumor cells of the explant, for they both resembled them and were in physical continuity with them. Since this growth did not resemble in any way any growths from normal or neoplastic smooth muscle cells, we feel justified in rejecting Dr. Pappenheimer's interpretation and adhering to our own, supported by our belief in the value of the evidence obtained from tissue culture.

A HERETOFORE UNRECOGNIZED MECHANICAL PRINCIPLE EFFECTIVE IN AORTIC SCLEROSIS

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From the point of view of clinical medicine the physical characteristics of the aorta are much more significant than are the histologic details. A series of studies based on the elastic properties of the aging aorta have led to a new theory of the role of mechanical factors in the causation of arteriosclerosis, which is correlated with the histologic picture (Krafka [1]). The technic used in making the analyses of elasticity is that of Roy [2] except that I have adapted the use of the Scott serigraph to the solution of this problem. The serigraph is a tensile strength-testing machine such as that used in rayon factories, in which the stretching force applied to the standard aortic strip increases from 0 to 250 Gm., as the attached weight, rolling on a movable track, varies its pull according to the formula ($F = W \times \sin A$), in which F is the force, W the weight and A the angle of the track with the horizontal.

In all of the material available to me the curve of elongation for the normal human aorta is an exponential curve, tending to straighten out at the maximum stresses. An analysis of the curve by the various methods indicates that within the range of greatest distensibility the elasticity is a function of (1) the elasticity of the elastic fibers, (2) the arrangement of fibers and (3) the variable tonus of the muscle fibers. In the straight line portion of the curve the moduli are comparable to those of a tendon and hence indicate that the collagenous fibers are acting as checks against overdistention.

That the interplay of the three histologic elements of the aorta give the characteristic curve is clear. A significant problem arises, however, as to whether or not the hollow of the curve represents the pressure range of normal systole and diastole.

At first glance, this would seem to be a relatively simple problem. But any attempt to resolve it into its principal factors immediately

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1. Krafka, J., Jr.: Arch. Path. **23**:1, 1937; Am. J. Physiol. **125**:1, 1939; Arch. Path. **29**:303, 1940.

2. Roy, C. S.: J. Physiol. **3**:125, 1880.

involves a complex study of mechanics, even if Love's twenty-one constants and Poisonn's ratio are neglected.

Resorting to the formulas of mechanical engineers which deal with stress in rigid thin-walled cylinders of homogeneous substances, at least fourteen separate methods of calculation may be developed, relating tension within the wall to hydrostatic force within the cylinder. I have discussed these formulas with my colleagues in the University of Georgia School of Medicine and with various physicists and engineers. I have selected two formulas for further consideration.

From the data previously published (Krafka, 1939) and from the accompanying figure it is definitely shown that the curve of elongation for strips of the aorta is an exponential curve with the hollow of the curve at the 25 to 100 Gm. tension level. Above this point the curve straightens out. The first impression one has on examining such a curve is that the systolic and diastolic pressure equivalents should fall somewhere on the hollow of this curve. This seems to be inherent in the logic of the situation; otherwise it is difficult to understand why the greatest variability in elasticity should occur below the range of adaptability to blood pressure. I realize that this concept is highly prejudicial. I have recognized its biasing influence and have deliberately sought an explanation outside it, but every rational analysis leads to the same end: *The mechanical equivalents of systolic and diastolic pressures do fall on the hollow of the stretch curve.*

The formula commonly used by engineers to determine stress in the wall of a thin cylinder is as follows:

$$(1) \qquad St = \frac{P \times D}{2t}$$

in which St is the stress, P the pressure, D the diameter and t the thickness of the wall of the cylinder. For the development of this formula the reader is referred to Maurer and Withey.[3]

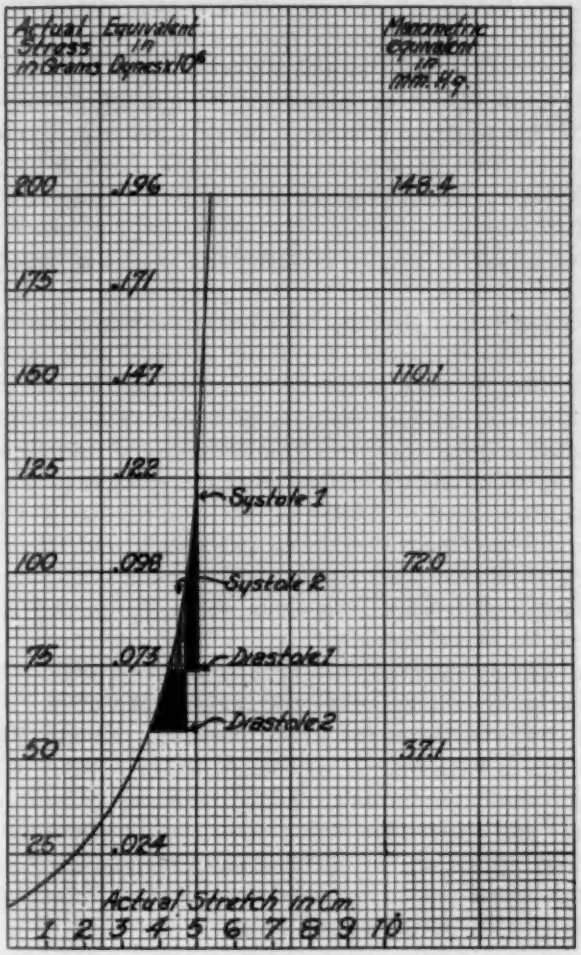
A second formula, independently developed, gives somewhat similar values.

$$(2) \qquad St = \frac{P \times Cm^2}{2}$$

in which P is the weight equivalent of a column of mercury, Cm^2 the surface area of the strip and 2 a corrective factor based on the hypothesis that the distending force is resolved into two vectors, one a longitudinal and the other a transverse, applicable since the stretch-strip method is applied in one direction only.

In order to convert the mechanical equivalents of the hydrostatic force represented by normal systolic and diastolic pressure into terms of tension as recorded by the stretching forces of the serigraph, all values have been recorded as dynes $\times 10^6$. Thus, at the left of the figure actual

3. Maurer, E. R., and Withey, M. O.: Strength of Materials, New York, John Wiley & Sons, Inc., 1935, p. 78.



Elongation curve for a 10 cm. longitudinal strip of a human aorta from a Negro woman aged 19 years, dead of Ludwig's angina. The blood pressure before death was 140 systolic and 90 diastolic. Actual stretch is indicated as abscissas; actual load, as ordinates. (Reduced one half in reproduction.)

Grams of stress (left) are converted to dynes $\times 10^6$ (center); also the mechanical equivalents of manometric values in millimeters of mercury are given (right). The upper blackened triangle shows the fixation points for systole and diastole as calculated by the formula

$$St = \frac{P \times D}{2t}$$

The lower triangle represents the fixation points as calculated by the formula

$$St = \frac{P \times Cm^2}{2}$$

Actual stretch between diastolic and systolic limits is indicated by the length of the base of each triangle.

stress as recorded on the serigraph is given in grams; at the right the blood pressure values, in millimeters of mercury, and in the center the mechanical equivalents of both, in dynes $\times 10^6$.

The conversion is based on the following concept. Ganot has given the value of 1 atmosphere of pressure as 760 mm. of mercury or 1.01327 dynes $\times 10^6$ per square centimeter. Hence 1 mm. equals 0.00132 dynes $\times 10^6$ and 100 mm. of mercury equals 0.132 dynes $\times 10^6$. Since a 200 Gm. stress for the serigraph is equal to 0.196 dynes $\times 10^6$, the two sets of values may be adjusted. The mechanical equivalent of a manometric value of 148.40 mm. of mercury is 200 Gm. as recorded by the serigraph.

A direct conversion of grams of linear stress to manometric pressure does not correctly express the relationship of hydrostatic pressure on the wall of the aorta, because of the nature of the application of the force. Dr. R. J. Roark, of the University of Wisconsin School of Engineering, has calculated for me the force equivalents in a specific case, using formula 1, in which the circumference of the aorta is taken at 61 mm., the cross-sectional area as 0.0853 sq. cm., the thickness of the wall as 1.598 mm. and the blood pressure as 140 systolic and 90 diastolic. By this method the systolic force equivalent is fixed at 0.1215 dynes $\times 10^6$, and the diastolic at 0.0733. These limits are represented in the figure by the upper blackened triangle.

My own calculations, based on formula 2, give a slightly lower set of values; namely, systolic at 0.0924 and diastolic at 0.0594. These limits are shown in the figure as the lower blackened triangle. The overlap of the two sets of values are cross-hatched in the figure.

While I consider the formula used by Dr. Roark as better established, I have made one observation which questions its use when applied to plastic hollow cylinders. On mathematical grounds, engineers consider that the longitudinal tension in a hollow cylinder is just half of that of the transi tension. The formula used is

$$S = \frac{P \times D}{4t}$$

They utilize this concept in a practical "rivet rule" in the construction of pressure tanks. In order to test this relationship in a plastic cylinder, the following observations were made. An automobile tube was inflated to turgor. A 2 inch (5 cm.) line was laid off in a transi direction and a second one in a longitudinal direction. The tube was then inflated further. The transi mark increased to 2⅜ inches (5.9 cm.), while the longitudinal mark stretched to 2⁷⁄₁₆ inches (6.1 cm.). Were the rivet rule operative in a plastic cylinder as in a rigid one, the transi mark should have shown an incremental increase twice that of the longitudinal. Evidently, equalization of force is accomplished by resistance in two directions. This point gains interest in the observed facts that longitu-

dinal strips and transverse strips of aorta show practically superimposed curves when stretched on the serigraph, and that the histologic elements in the aorta are disposed in a grid fashion.

The more significant point, however, is that by either of the methods of calculation used in the foregoing paragraphs, the systolic and diastolic force equivalents fall well within the hollow portion of the elongation curve.

As a further check on this concept, considerations of stroke volume may next be undertaken. I have previously discussed the postulated stretch requirements of the aorta as based on the work of Roy (Krafka, 1937). From the figure it will be seen that for a 10 cm. strip the actual stretch between the diastolic and systolic values is 0.9 cm., or 9 per cent. This is consistent with a 26 per cent stretch from 60 to 130 mm. as calculated from the data of Roy, since the proportional value for 90 to 140 mm. would be 18.5 per cent for both longitudinal and transverse directions, and this halved would give 9.25 per cent for the longitudinal only.

If the stretch is measured on the curve for the 120 to 200 mm. interval, the observed stretch for these higher pressures is 5 mm., or 5 per cent, for the 10 cm. strip, again consistent with the observed values of Roy of 4.5 per cent when closed segments are filled under pressure.

With the establishment of the systolic-diastolic positions on the stretch curve, significant considerations of the mechanics of the aorta now appear. I have previously developed the thesis that it is at the stress levels represented by the hollow portion of the curve that the smooth muscle acts (Krafka, 1939). A strip in which the muscle is physiologically active gives a shallower curve than one in which the muscle is relaxed. A relaxed aorta subjected to the action of ephedrine shows a decrease in the hollow of the curve. The action of muscle in the hydrostatics of the aorta becomes self evident. With contraction of the muscle in the aortic wall, distensibility is decreased, aortic volume is reduced and blood pressure necessarily rises.

On the basis of the foregoing considerations, a study on the relation of the elasticity of the aorta in fibrosis and arteriosclerosis to blood pressure has been made, and a new concept of the loss of elasticity has been developed on the analysis of some 50 human cases (Krafka, 1940).

The present study directs attention to one more concept which has escaped investigators in the past. As shown in the foregoing discussion, the rivet rule is not operative in a plastic cylinder such as the aorta. With aging and with fibrosis and sclerosis, *there is a tendency to convert the plastic cylinder into a rigid cylinder*. Under these conditions the rivet rule again becomes operative and the hydrostatic force is resolved

into its two vectors, transi and longitudinal, in their mechanical relations of two to one. Hence *pressures which normally would fail to produce rupture are now redistributed with dire results.*

SUMMARY

By using the formulas of mechanical engineering for stress in the walls of thin-walled cylinders, it is possible to relate stress in the aortic wall to blood pressure values.

Calculated values for systolic and diastolic pressures fall well within the hollow portion of the exponential curve obtained by stretching strips of aortas on the serigraph.

Percentage extensibility of the strips of aortas within the stress equivalents for systolic and diastolic pressures is consistent with the normal stroke volume considerations.

With aging, fibrosis and sclerosis there is a tendency to convert the plastic cylinder into a rigid one. A redistribution of force vectors accordingly follows the rivet rule, and hence a normal blood pressure may become an effective agent of rupture.

ELASTIC TISSUE

II. A STUDY OF THE ELASTICITY AND TENSILE STRENGTH OF ELASTIC TISSUE ISOLATED FROM THE HUMAN AORTA

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In a previous report several qualities of purified elastic tissue isolated from the human aorta were discussed.[1] The qualities of extensibility, retractility and tensile strength were such that they could be conveniently measured by quantitative methods. The present report is concerned with the methods by which the maximum extensibility, minimum retractility and tensile strength of purified elastic networks are compared with the corresponding properties of the aortic walls from which the networks were isolated.

METHODS

The description of methods may be divided into four parts: first, the preparation of the fresh aorta for study; second, the determination of the quantity of elastic tissue in the aorta; third, the measurement of extensibility, retractility and tensile strength of the fresh aorta and of the purified elastic networks, and fourth, the evaluation of the tensile strength of elastic tissue.

Preparation of the Aorta for Study.—Twenty-one human aortas, varying in age from 10 days to 77 years, were obtained post mortem in the fresh state. The thoracic aorta was resected, and after a large part of the adventitia had been removed, was divided at the level of the second intercostal artery. The proximal segment was set aside for study of the quantity of elastic tissue. The distal segment was divided transversely into a series of cylindric rings which varied from 4 to 5 mm. in height. These rings were available for morphologic and physical studies.

Determination of the Quantity of Elastic Tissue in the Aorta.—The proximal segment of each aorta was reduced to dryness by successive treatments with alcohol, ether and heat (110 C.). After cooling in a desiccator, each dry segment was weighed. The segment was then rehydrated and placed at 45 C. in a volume of 89 per cent formic acid equal to 1 cc. for each 5 mg. of dry tissue. After twenty-four hours' extraction, the segment was washed to neutrality, reduced to dryness as before and weighed. Following this treatment, extraction was continued, and the loss of weight was again estimated at the termination of forty-eight and seventy-two hours' extraction. The value at the end of seventy-two hours, at which time the residue was composed essentially of elastic tissue,[1] was taken as an arbitrary standard value of purification.

From the Department of Pathology of Cornell College of Medicine.

1. Hass, G. M.: Arch. Path. **34**:807, 1942.

Measurement of Extensibility, Retractility and Tensile Strength of Aortic Segments.—The series of cylindric rings of each fresh aorta were approximately of equal dimensions with a variation of 1 to 3 mm. in circumference and 0.1 to 0.3 mm. in breadth. They were used for four different purposes. Those which did not conform to the average physical behavior were cut so that the average circumference of the series could be computed from linear measurements of the strips. Those which conformed to the average physical behavior were divided into pairs. One pair was fixed at once for histologic study. Three or more pairs were set aside for extraction. After extraction with 89 per cent formic acid for twenty-four hours, one pair was washed to neutrality. Of the two, one was fixed for microscopic study. The second was subjected to measurements of extensibility, retractility and tensile strength. After rupture, the circumference of the segment was measured linearly. Then the segment was reduced to dryness and a measurement made of its minimum breadth. This procedure was repeated on pairs of aortic rings after extraction for forty-eight and seventy-two hours.

The apparatus for estimating the response of fresh and extracted aortic segments to application and removal of loads may be described as follows: It consisted of an upper rigid steel pin, a lower mobile steel pin with a hook to which weights could be attached, an adjustable millimeter rule and a horizontal platform which could be moved vertically by rotation of a screw.

The cylindric ring of aortic wall was suspended on the upper rigid pin and placed under tension of a load of 5 Gm. The millimeter rule was adjusted and a zero reading was made. The load was increased at increments of 5 or 10 Gm. up to a maximum load of 120 Gm. Thereafter, increments of 50 Gm. were applied except when isolated elastic networks were being studied. In these instances increments of 5 or 10 Gm. were used until the networks ruptured. After the application of each load, the extension was measured and after the removal of each load, the failure to retract to the zero reading was measured.

Near maximum extension of most fresh segments was reached at loads of 220 Gm. This value, converted into percental extension in terms of the original circumference, was accepted as an arbitrary standard for the fresh aorta. This value, for any given vessel, was approximately the same for all selected segments.

Near maximum extension of most segments of pure elastic tissue was reached at variable loads. Usually this extension was measurable on application of the breaking load. At other times the rupture under tension was so rapid that the value for maximum extension was at a load 5 or 10 Gm. less than the breaking load.

Near minimum retractility of the fresh segments was estimated after removal of the load of 220 Gm. The difference between the final and the initial circumference under tension of 5 Gm. divided by the initial circumference was selected as a standard expression for retraction.

Near minimum retractility of isolated elastic tissue was estimated as the percentage of permanent elongation of the original circumference of the segments. This value was obtained from measurements after removal of a load which was 5 or 10 Gm. less than the breaking load.

The breaking loads for fresh aortic segments were far in excess of applied loads, so that the tensile strengths of the intact aortic walls are unknown.

The breaking loads of the elastic networks were always accurate to the nearest 5 or 10 Gm.

Evaluation of the Tensile Strength of Isolated Elastic Systems.—The tensile strength of the purified elastic tissue was determined at maximum extension under the tension of a breaking load. This determination required a knowledge

of the resting length of the segment, the maximum elongation under tension of the breaking load, the minimum breadth of dry segments after seventy-two hours' extraction, the minimum thickness of the dry intact aortic wall, and the percentage of the dry intact aortic wall in the form of elastic tissue.

The means for determining the original circumference of the segment, the maximum extension of isolated networks and the breaking load have been described.

The minimum breadth of the extracted aortic ring was measured to the nearest 0.1 mm. after rupture of the elastic system under tension and after desiccation of the flat strip of elastic tissue at zero tension.

The minimum thickness of the dry intact aortic wall was evaluated from a study of microscopic preparations of control contiguous rings. These preparations, stained by Weigert's method for the demonstration of elastic tissue, were magnified one hundred times with a photomicrographic apparatus. Direct measurements of the minimum thickness of the aortic wall from the endothelium to the external elastic lamellas of the media were made on the image projected on a ground glass screen.

The following formula was used for calculating the tensile strength of elastic tissue:

$$\left.\begin{array}{c}\text{Tensile}\\ \text{strength}\\ \text{in grams}\\ \text{per square}\\ \text{centimeter}\end{array}\right\} = \frac{\text{breaking load in grams}}{2 \times \begin{array}{c}\text{original}\\ \text{circum-}\\ \text{ference}\end{array} \times \begin{array}{c}\text{minimum area}\\ \text{of dry cross}\\ \text{section}\end{array} \times \% \begin{array}{c}\text{elastic}\\ \text{tissue}\end{array}}{\text{circumference at maximum extension}}$$

The assumptions which enter into this calculation are obvious and need not be enumerated. In the average case the errors are not great even if the assumptions are incorrect. In individual cases the error may be great because the point of maximum weakness may not coincide with the point of near minimum dimensions. A careful study may be planned so that cross-sectional areas at planes of rupture may be evaluated. I do not know how this can be done, unless measurements of the area at the plane of transverse rupture are known before the breaking load is applied.

RESULTS

Comparison of the Values for Near Maximum Extensibility of the Intact Aorta and Isolated Elastic Tissue.—The values recorded in column 4 of table 1 show that the maximum extensibility of the normal aorta is low in infancy. There is an average increase of extensibility during the first three or four decades of life. Thereafter, there is an average decrease so that the values in the eighth decade are slightly less than those in the first few days of life.

The values recorded in column 5 of table 1 were obtained by measurements of the maximum extensibility of elastic networks isolated from those segments whose measurements as intact vascular structures are recorded in column 4.

The average maximum extensibility of the isolated elastic tissue is 32 per cent greater than that of the vascular walls from which it was obtained. The infant elastica has the greatest extensibility. This is in contrast to the low order of extensibility of the intact vessel. With increasing age there is an average progressive decline in the percentage of maximum extensibility. Hence, sometime in the early decades of life the intact aorta is so constructed that then and only then is the full extensibility of the networks utilizable. In the young and the aged intact vessel the potential motion of the networks is under constraint.

TABLE 1.—*Extensibility of the Aorta and of the Elastic Tissue Isolated from It*

Number	Age, Yr.	Circumference of Aorta, Cm.	Maximum Extension,* per Cent		Failure to Retract,† per Cent	
			Fresh Aorta	Elastic Tissue	Fresh Aorta	Elastic Tissue
24	.028	1.6	41.4	87.9	4.4	3.8
38	12	3.5	74.1	54.7	11.4	1.7
55	28	3.8	48.0	71.8	11.6	3.8
42	35	5.0	68.8	57.1	8.0	3.4
41	41	5.1	51.2	51.7	7.1	2.4
52	42	4.5	55.1	67.5	3.1	3.1
34	44	4.6	59.8	56.0	7.6	4.2
39	45	5.1	55.2	74.6	9.8	4.9
53	50	4.8	58.4	52.2	6.7	1.7
47	51	5.2	55.4	63.1	8.5	4.1
26	53	4.7	42.8	62.2	16.2	4.4
45	53	5.4	52.2	61.8	8.9	4.5
27	54	4.9	55.0	57.0	10.2	4.7
43	54	6.0	45.3	42.5	10.0	1.4
40	58	5.4	49.2	44.8	11.1	1.5
51	58	5.3	37.7	62.3	7.6	4.3
48	62	6.2	42.0	42.5	10.3	3.4
25	63	4.7	38.0	60.0	9.6	1.0
31	70	5.7	29.7	70.0	7.9	3.9
46	71	7.2	38.1	44.9	8.3	6.3
49	77	6.8	33.6	39.1	11.8	3.2
Average	49	5.0	44.3	58.3	9.1	3.4

* This means the extension of the fresh aorta under a 220 Gm. load (near maximum extension) and the extension of the elastic networks at a breaking load.

† This is calculated in terms of the circumference of the fresh aorta and of the isolated neutral elastic networks; near equal values.

Though the average extensibility of pure networks declines with age, there are interesting individual exceptions. For instance, the extensibilities of isolated elastica at ages of 0.028, 28, 45 and 70 years are of a similar order of magnitude, which approximates maximum values. However, all systems with a low order of extensibility have an age of at least 54 years. It is likely that a larger series, with emphasis placed on the younger age groups, will disclose some systems of low extensibility, because it is clear that extensibility is not an exclusive function of age or the morphologic change known as atherosclerosis.

The sources of error in the measurement of extensibility are not negligible. The principal error is due to the variable rigidity of the cylindric segment. In measurements of older, rigid segments, the tare

weight of 5 Gm. does not change the cylindric contour to the degree observed when measurements of plastic, younger segments are made. Under these circumstances the measured elongation of the rigid vessel is more than the actual elongation. This source of error is not encountered when measurements are made of isolated elastic systems because they have little tendency to retain cylindric contour under a tension of 5 Gm. Therefore, the measurements of the extensibilities of pure elastic systems at all ages are carried out with negligible error, while errors in dealing with intact aortas are much greater. It would be desirable to measure strips rather than cylinders, but the technical difficulties in handling strips of purified elastic tissue are too great.

Comparison of Values for Near Minimum Retractility of the Intact Aorta and Isolated Elastic Tissue.—The failure of the fresh aorta to retract after removal of the maximum load of 220 Gm. is expressed in percentage of the original circumference. The figures are recorded in column 6 of table 1. In column 7 of the same table there is a corresponding set of figures which pertain to the retractility of unconstrained isolated networks on release of loads producing near maximum extensions.

These data show that in every instance the retraction of purified elastic tissue is more complete than the retraction of the aortic wall from which it was isolated. The average aortic segment undergoes a permanent elongation of 9.1 per cent after near maximum extension. The elastic system of the average aortic segment undergoes a permanent elongation of only 3.4 per cent even though the value for near maximum extension is usually in excess of that of the intact aortic segment.

The retractility of the intact aorta is slightly reduced with increasing age. The retractility of the isolated elastic networks is as complete in the case of the old as in that of the young person.

In individual cases the retraction of pure elastica is nearly complete, and within limits of error the tissue possesses perfect elasticity up to maximum extension and rupture.

The errors in measurement of retractility are essentially the inverse of those which apply to measurements of extension. The principal errors are due to the variable rigidity of rings and variable permanent deformation of the circular contour of the rings after removal of load. It is improbable that the errors are great enough to modify the conclusions drawn from the data.

Results of Measurements of Dimensions.—The minimum thicknesses of the dry aortas as measured from the endothelium to the external medial networks are recorded in column 3 of table 2. The average thickness increases gradually with age. The lowest value, 0.053 cm., is a measurement of a 10 day old aorta, and the highest value, 0.182 cm., is a measurement of a 44 year old aorta.

The minimum breadths of the dry aortic strips are recorded in column 4 of table 2. These are relatively constant. The average minimum breadth is 0.395 cm.

The errors involved in measurements of minimum thickness and breadth are small. The use of the data obtained by measurement of a control segment in the estimation of a dimension of a contiguous experimental segment is an uncorrected source of error. The data with respect to breadth may not necessarily represent the minimal breadth of the experimental segment because the experimental rings were ruptured before the data were obtained.

TABLE 2.—*Measurements of Dimensions and Tensile Strength*

No.	Age, Yr.	Minimum Dimensions (Dry), Cm.* Thickness	Breadth	Maximum Extension, per Cent	Elastin, per Cent	Cross Section † of Elastin, Sq. Cm.	Breaking Load, Gm.	Tensile Strength, Gm. per Sq. Cm.
24	.028	0.053	0.38	87.9	28.9	0.0058	30	4,870
38	12	0.074	0.36	54.7	40.6	0.0108	70	5,040
55	28	0.092	0.37	71.8	41.1	0.0140	110	6,750
42	35	0.140	0.40	57.1	40.2	0.0225	90	3,140
41	41	0.118	0.38	51.7	40.4	0.0181	80	3,360
52	42	0.110	0.41	67.5	40.9	0.0184	120	5,450
34	44	0.182	0.41	56.0	30.7	0.0229	70	2,400
39	45	0.137	0.42	74.6	34.2	0.0197	140	6,220
53	50	0.117	0.39	52.2	38.2	0.0174	80	3,490
47	51	0.135	0.38	63.1	40.2	0.0206	100	3,950
26	53	0.146	0.40	62.2	37.4	0.0218	70	2,600
45	53	0.130	0.37	61.8	36.3	0.0175	110	5,090
27	54	0.124	0.39	57.0	38.5	0.0186	80	3,380
43	54	0.142	0.40	42.5	42.2	0.0240	50	1,490
40	58	0.143	0.42	44.8	30.1	0.0181	50	2,000
51	58	0.128	0.44	62.3	41.9	0.0236	110	3,790
48	62	0.148	0.41	42.5	41.7	0.0253	70	1,970
25	63	0.150	0.37	60.0	40.7	0.0226	50	1,770
31	70	0.155	0.42	70.0	41.5	0.0270	110	3,450
46	71	0.140	0.38	44.9	31.1	0.0165	80	3,490
49	77	0.150	0.40	39.1	39.0	0.0234	50	1,490
Aver.	49	0.129	0.395	58.3	37.9	0.0194	82	3,580

* These are the measurements of the dry vascular wall at zero load.
† The cross-sectional area of a vascular segment × 2 = the area at zero load.

Estimation of Quantity of Elastic Tissue.—The quantities of elastic tissue in the several aortas are recorded in column 6 of table 2. The percentages of the vessels recovered as elastic tissue vary from 28.9 to 42.2. The average value of 37.9 per cent is higher than the value of 30 per cent which has been reported by other workers.[2]

Though the degree of impurity of the elastic tissue isolated by the present method is unknown, it is certain that the order of impurity is almost constant in all preparations and that the impurities do not influence measurements of physical properties of the isolated elastic systems.

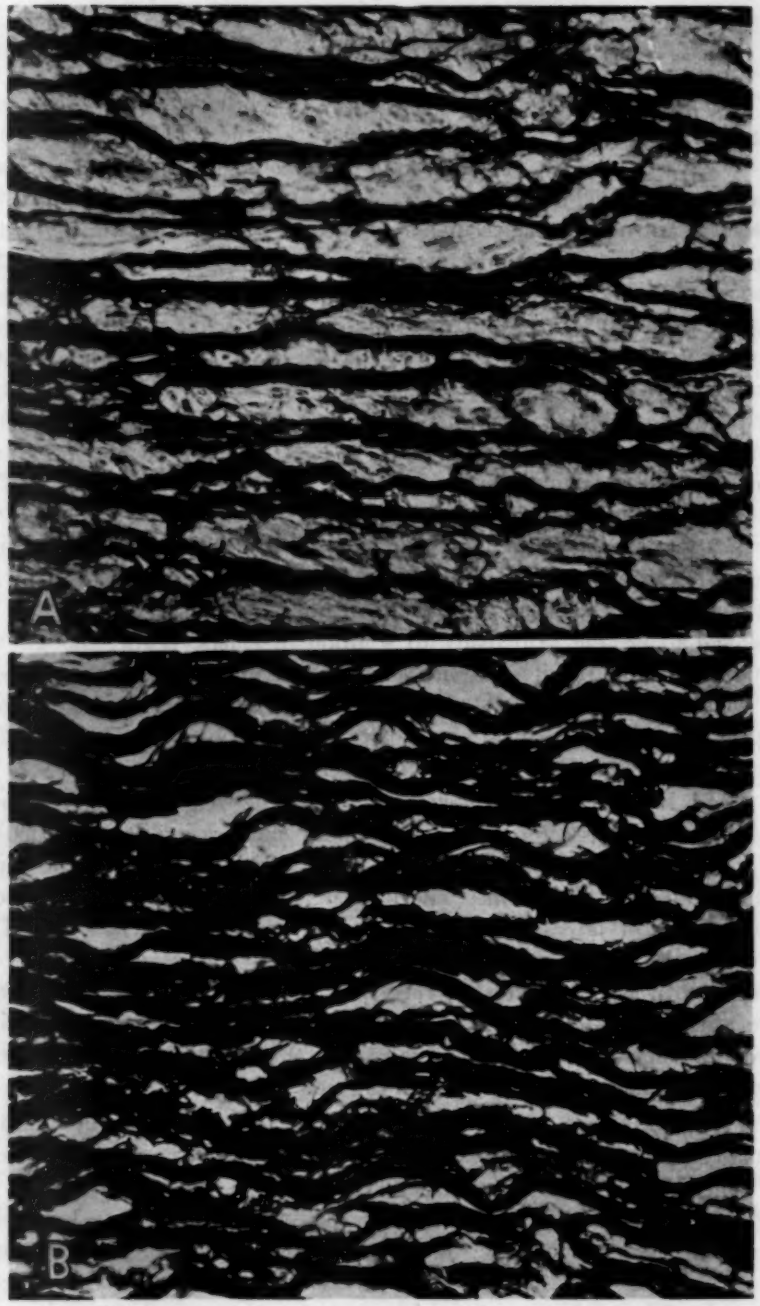
2. Lowry, O. H.; Gilligan, D. R., and Katersky, E. M.: J. Biol. Chem. **139**:795, 1941.

There is no correlation between the age of the aorta and the percentage of the vessel in the form of elastic tissue. It is apparent that in the average case an increase in the dimensions of the aorta is accompanied by a parallel increase in the amount of elastic tissue. In individual cases this is not true, presumably because of undue increases in the amount of collagen in the intima or the media. Three variations of this type are shown at 44, 58 and 71 years of age.

The principal source of error in these studies is due to absence of two sets of data: first, the exact rate of degradation of elastica in the seventy-two hour extraction interval and, second, the exact degree of purity of each elastic system. The rate of solution of elastic tissue under the conditions used for purification is 2 to 5 per cent in each twenty-four hours after the termination of seventy-two hours of extraction. The rate in the interval up to seventy-two hours is unknown. The rate of purification is rapid in the interval up to twenty-four hours, slower in the twenty-four to forty-eight hour interval and essentially constant for all networks at the end of seventy-two hours. This constant rate is assumed to be due principally to solution of elastic tissue, though the removal of some residual impurities may contribute to the observed loss of weight in the seventy-two to one hundred and sixty-eight hour extraction intervals. Absolute purity is probably unattainable. For present purposes absolute purity is not required.

Loads Which Rupture Elastic Systems.—The weights which ruptured the elastic systems after seventy-two hours' purification are recorded in column 8 of table 2. The breaking loads varied from 30 to 140 Gm., with an average of 82 Gm. The loads among the several vessels were independent of any factor except tensile strength of networks. The errors in determination of the breaking loads were insignificant, but in dealing with a series of elastic rings isolated from an aorta with extensive atherosclerotic changes, considerable differences between breaking loads for rings of similar dimensions were encountered. The minimum and maximum values in any series did not differ more than 30 Gm.

Tensile Strength of Purified Elastic Networks.—The values for the tensile strength of isolated elastic tissue are recorded in the last column of table 2. They vary from 1,490 to 6,750 Gm. The average of 21 aortas is 3,580 Gm. As a rule, the tensile strength decreases with increasing age. There are several important exceptions. The aorta of a patient 44 years of age possessed networks of unusually low tensile strength. The aorta of a patient 53 years of age possessed elastic systems of unusually high tensile strength. Illustrations of the wall of this aorta before and after purification of elastic networks are shown in the accompanying figure. Other individual variations which do not conform to the general rule are recorded in table 2.



A, Weigert-Van Gieson preparation of the media of aorta 45, aged 53. Note the separation of elastic lamellas by abundant nonelastic tissue structures. Compare this illustration with *B*.

B, Weigert-Van Gieson preparation of the media of the aorta in *A* after extraction of all components except elastic tissue. The elastic lamellas of this aged vessel were of good quality with a tensile strength of 5,090 Gm. per square centimeter.

The tensile strength of networks obtained from a vascular segment is independent of the percentage of elastic tissue in the segment. Low values for tensile strength are encountered at near minimum and maximum values for percentage of elastic tissue, namely, 30.1 and 42.2. High values are likewise found at near extremes of the range of percentage of elastic tissue, namely, 28.9 and 41.1.

There is a common parallel relation between tensile strength and extensibility of isolated elastic networks. Networks with low tensile strength usually have a low range of extensibility, while elastic systems of high tensile strength have high average maximum extensibility. Tensile strength and extensibility are two good criteria by which the true quality of the elastic tissue may be judged.

There is no relation between tensile strength and capacity of isolated elastic systems to retract after removal of maximum tolerated loads. However, other data, unrecorded here, show that at similar percental extensions, the elastic systems of high tensile strength possess greater retractile power than those of low tensile strength.

Values for tensile strength are derived by calculations which involve several quantities. These are, first, the minimum thickness of the dry intact aorta; second, the minimum breadth of dry strips of purified elastica; third, the percentage of the aortic wall recovered as elastic tissue; fourth, the percentage near maximum extension; and fifth, the breaking load at near maximum extension. The tensile strength is referred, therefore, to the minimum dry cross-sectional area of a solid mass of elastic lamellas at near maximum extension under the tension of a breaking load.

The calculation of tensile strength is no more accurate than the measurements from which the calculation was made. It is, perhaps, less accurate, because it is necessary to introduce three assumptions. First, it is assumed that regions of minimum breadth and thickness coincide. In general, this is not true. Second, it is assumed that the average percentage of elastic tissue in the vessel is the percentage of elastic tissue at the area of minimum breadth and thickness. In general, this is not strictly true. Third, it is assumed that rupture occurs at regions of minimum breadth and thickness in areas which have an average percentage of elastic tissue. This is not a uniformly dependable assumption, especially when pathologic changes have altered the structure of the vascular wall. Finally, it is assumed that the lamellas and fibers decrease in a cross-sectional area in direct proportion to the increment in length on extension. The correctness of this assumption depends on the intrinsic structure of the networks. If the intrinsic structure, for instance of lamellas, is a coiled or angulated meshwork of fibers, such as those illustrated in a previous report, the decrease of dimensions on extension is different from the calculated decrease.

The values for tensile strength, with due regard for the errors in measurement, are not absolute values. Native elastic tissue was not studied. All networks were subjected to conditions which diminish natural tensile strength. The precise degree of this decrease is unknown. The rate of decrease in each twenty-four hour extraction interval, however, has been measured. From these unrecorded data it may be concluded that if the decrease of tensile strength up to ninety-six hours of extraction is a linear function of time, the observed reduction in tensile strength in the interval is in the average case almost directly proportional to the estimated decrease in the cross-sectional area of the networks. From this it may be inferred that the tensile strength of native systems is not much greater than that of the chemically isolated systems. An immediate unsustained action of formic acid on the tensile strength of elastic tissue is quite probable. If this occurs, the foregoing inference is not justified.

COMMENT

Though the errors of the quantitative study of the physical properties of purified elastic systems are at times intolerable, it is hoped that the numerous defects in method may eventually be eliminated so that more reliable data may be obtained. In its present form, the method is suitable for the detection of elastic systems of variable quality. Such a segregation of systems is impossible by sole recourse to the classic methods of physical or morphologic study of the intact aorta. Nor do the disclosed properties of isolated systems conform to properties attributed to these systems by study of the intact vessel. From the standpoint of disease of the vascular system or other tissues to which the method may be applied, elastic networks of low tensile strength seem to present the most interesting problems.

SUMMARY AND CONCLUSIONS

In a series of 21 human aortas, aged 10 days to 77 years, the amounts of elastic tissue which were recovered varied from 28.9 to 42.2 per cent, with an average of 37.9 per cent.

The quantity of elastic tissue in each unit volume of the average aortic wall remains nearly constant throughout life. Individual variations are included in the range 28.9 to 42.2 per cent.

The purified elastic systems possess an average of 32 per cent greater extensibility and 170 per cent greater retractility than the intact aortic walls from which they are isolated.

The average maximum extensibility of isolated elastic tissue decreases with increasing age in a manner which cannot be predicted by a study of the intact aorta.

The retraction of isolated elastic tissue after extension is always more complete than that of the intact vessel. The magnitude of retraction is the same for all isolated networks and is independent of their age.

The tensile strength of isolated elastic tissue varies from 1,490 to 6,750 Gm. per square centimeter of dry cross-sectional area at maximum extension. In general, tensile strength decreases with increasing age. There are several unexplained exceptions to this average rule.

ATROPHY, DEGENERATION AND METAPLASIA IN DENERVATED SKELETAL MUSCLE

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The atrophy of skeletal muscle that follows separation of the latter from the motor neuron can be investigated by various methods, such as microscopic and chemical methods, and by the determination of the loss of weight.

The material on which the research reported here is based consisted of 8 cats and 11 rabbits, in each of which the sciatic or the lateral popliteal nerve was cut. Forty to one hundred and thirteen days were allowed to elapse before the animals were killed. In every case the denervated muscle was weighed and its weight compared with that of the muscle of the opposite (normal) side. The tissue was fixed in Susa's fluid and stained by various methods, such as Delafield's hematoxylin-eosin, iron-hematoxylin-eosin, Mallory's acid hematoxylin, Mallory's aniline blue–orange G and Foot's reticular stain.

The loss of weight of muscles as the result of atrophy is still regarded as an outstanding sign. It is often taken as the only measurement for determining the extent of atrophy. This loss of weight taken by itself, however, is not a reliable sign, as it does not give any indication of what is actually happening to the muscle element. Besides, it should be kept in mind that muscle atrophy and degeneration depend not only on the duration of the atrophic process but also on the natural longevity of the species of animals concerned (Knowlton and Hines [1]) and the particular muscle involved (Langley and Hashimoto [2]; Tower [3a]).

The appearance of atrophic muscle is clearly shown in figure 1. It is evident that the replacing connective tissue is white fibrous in some cases and predominantly adipose or a mixture of the two in others. Thus the denervated musculus semitendinosus of a rabbit (fig. 1 *A*) reveals a predominance of adipose tissue replacing the muscle fibers,

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From the Department of Anatomy of the University of Saskatchewan.

This work was made possible by a grant from the National Research Council of Canada.

1. Knowlton, G. C., and Hines, H. M.: Proc. Soc. Exper. Biol. & Med. **35**:394, 1936.

2. Langley, J. N., and Hashimoto, M.: J. Physiol. **52**:15, 1918.

3. Tower, S. S.: (*a*) Am. J. Anat. **56**:1, 1935; (*b*) J. Comp. Neurol. **67**:241, 1937; (*c*) Physiol. Rev. **19**:1, 1939.

with a loss of weight of 46 per cent, while the atrophied anterior tibial muscle (fig. 1 *B*) shows a predominantly fibrous tissue replacement, with a loss of weight of 64 per cent. Other muscles have shown a

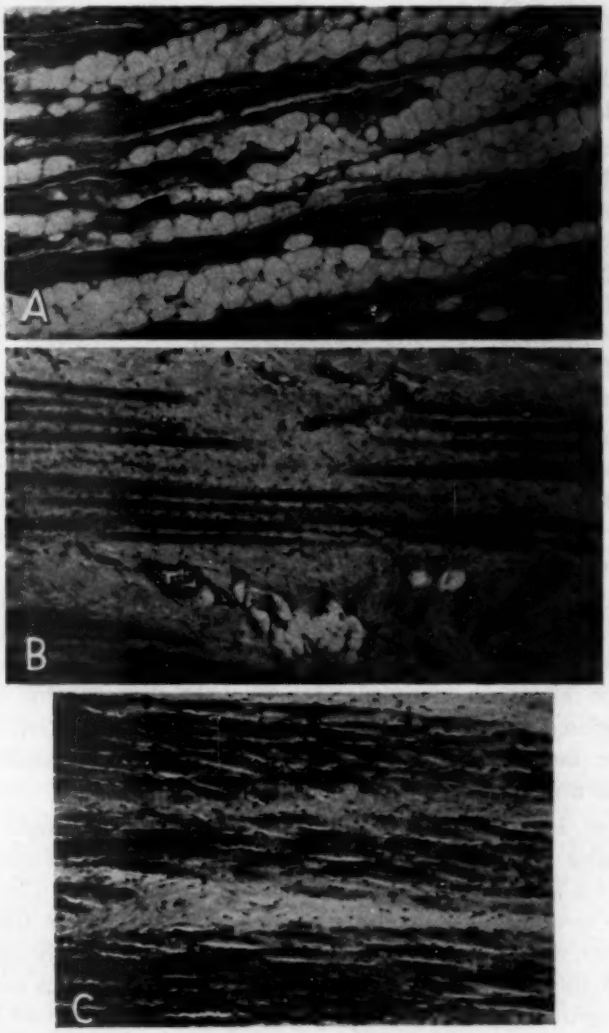

Fig. 1.—*A*, musculus semitendinosus of rabbit X. Susa fixation; paraffin section; Delafield's hematoxylin-eosin; × 73. *B*, musculus tibialis anterior of rabbit V. Technic and magnification as in *A*. *C*, musculus tibialis anterior of the same animal as the muscle in *A*. Technic and magnification as in *A*.

marked loss of weight with little increase in any of the connective tissue elements. Such a muscle is shown in figure 1 *C*; the loss was 65 per

cent. Here the muscle fibers, diminished in size, are placed close together. The type of reaction of the connective tissue is not dependent on a difference in species or on the muscle involved, since the aforementioned forms of reaction were found in different muscles of the same animals. On the other hand, the same type of muscle reacted differently in several animals.

For these reasons it seems wise to abstain from generalizing results and to limit statements as much as possible.

A well known change in atrophic muscles is the great increase in the number of subsarcolemmal nuclei. This increase is not only a relative one, due to the decrease in volume of the parenchyma, but also an absolute increase. The proliferation of nuclei, according to some authors (Chor, Dolkart and Davenport [4]) is due to mitosis, but others (e. g., Tower [3c]) have considered it due to amitosis. In my own observations, made on the semitendinosus, the extensor digitorum longus, the tibialis anterior and the gastrocnemius of cats and rabbits, the only form of nuclear proliferation was by amitosis.

The increase in number of the subsarcolemmal nuclei is believed to be caused by toxic stimulation from products liberated from the degenerating nerve tissue (Tower [3b]). This explanation corresponds to one of those that have been given for the reparative proliferation that follows destruction of tissue and even for tumor growth.

The nuclear proliferation could be interpreted teleologically as an attempt toward regeneration. But not even a trace of actual repair has been found, and such a theory, though suggestive, can be only an assumption based on mere speculation about the economy of nature and a supposed scope of the numerical increase of those nuclei. Furthermore, the factor actually causing the nuclear proliferation remains obscure in this explanation.

Another explanation merits some consideration, though it is not demonstrable in a direct way. The increase in number of nuclei might be due to a loss of equilibrium of tension in the tissue. In the case of muscle atrophy there would be decreased tension inside the atrophic muscle fiber, due to waste of some of its constituents, mainly sarcoplasm. Such a disturbance of tissue tension has been assumed in explanation of tumor growth, though in that case the disturbance would be rather intercellular than intracellular, as in the muscle fiber. Diminished muscle tension in the muscle fiber has been invoked as causing the nuclei of tadpole muscles to become round (Speidel [5]).

4. Chor, H.; Dolkart, R. E., and Davenport, H. A.: Am. J. Physiol. **118**: 580, 1937.

5. Speidel, C. C.: Am. J. Anat. **62**:179, 1938.

A related explanation for the formation of nuclear rows in heart muscle fibers has been attempted by Schiefferdecker,[6] who, according to Häggqvist,[7] considered "the nuclear rows rather as a sign that the equilibrium was disturbed in the muscle fibers, a fact which, in adults might be connected with diseases of which they had died, and in children with growth."

The often encountered central position of the nuclei in many atrophic fibers could be explained as dedifferentiation of the muscle fiber and return to the more primitive stage of the red muscle. It could also be explained as regression toward the early premature stage. But these explanations are mere speculations or comparisons on a purely morphologic basis and should not be overestimated or overemphasized.

Another possible explanation is that the few centrally placed nuclei normally found increase very much by division and that such nuclei are thus to be considered not as displaced but as autochthonous and merely numerically increased. In cross sections there is some evidence that subsarcolemmal nuclei are migrating toward the center of the fiber, but it seems impossible to demonstrate that such a nucleus observed and localized in a cross section corresponds to a nuclear row, and it can only be conjectured that it would have arrived in the center if the animal had survived.

The partial replacement of muscle parenchyma by connective tissue is brought about by two factors. One of them is wasting of the muscle fiber with proliferation of the surrounding connective tissue. The other factor is transformation of muscle fibers into connective tissue fibers by way of metaplasia. This has been explicitly described by Tower, and in many instances I have been able to follow the process in its various stages.

The process of metaplasia in muscle may occur in different parts of the muscle fiber. In some cases it appears to attack the pole of the fiber transforming the myofibrillae into collagenous fibrils, while elsewhere the process occurs in the body of the fiber, causing complete interruption and producing a collagenous bridge which divides the fiber into two parts. While in such cases the metaplastic process shortens the muscle fiber proper, in other instances, according to my observation, the metaplastic process starts along one side of the fiber, diminishing thus its small diameter. Such changes are seen distinctly in cross sections.

The transformation of myofibrillae into connective tissue fibers is often visualized especially well with Mallory's aniline blue stain, in which

6. Cited by Häggqvist.[7]

7. Häggqvist, G., in von Möllendorff, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1931, vol. 2, pt. 3.

the transformed fibrillae are a mere prolongation of the preserved myofibrillae but lack the cross striation and stain deeply blue, in contrast to the reddish purple muscle tissue.

The replacing fat tissue, which is sometimes conspicuous, seems in some instances to be built at the expense of the fibrous tissue, which in its turn was formed by metaplasia from striated muscle. In other places the adipose tissue arises in the interstitial connective tissue.

Insufficient attention has been paid to the reaction of the nuclei in such cases of metaplasia. The subsarcolemmal nuclei have been said to undergo different changes, such as swelling, loss of nucleoli, progressive slimming and fragmentation, and their ultimate fate, it has been claimed, is complete disappearance. Though this is true for many nuclei, a considerable number of them undergo metaplastic changes. Some features of these have been noted by Tower,[8a] who wrote: "During this metamorphosis, the nuclei which are caught between the fibrils resume an elongated form, but retain their position, resembling then, tendon nuclei."

In my observation the nuclear changes involved in metaplasia of muscle tissue are even more extensive. In many of them the nucleoli become inconspicuous or disappear. The nuclei decrease in size, especially in width, become darker and often bent, and their membrane shows one or at most a few distinct longitudinal folds. In the meantime, first at one nuclear pole and later at the other, a faintly staining basophilic substance appears, sometimes displaying the form of a cap, but at other times showing less distinct features (fig. 2). This faint plasmatic substance darkens, thins out and in some instances forms a cilium-like structure. This may in turn be drawn out into a long dark process placed parallel to and between the connective tissue fibers that correspond to the former myofibrillae. In most cases a thin layer of the newly acquired plasmatic substance is seen to surround the nucleus between its poles.

The polar processes of some nuclei are bifurcated or even more ramified. Because of the bending of the nuclei and the folding of the nuclear membrane, some of the nuclei assume bizarre forms. But the final appearance of the majority of these transformed nuclei with their perikaryon is that of fibroblasts, such as those found in coarse organized connective tissue, e.g., in fibrous capsules and fasciae.

So far I can give no final explanation as to the appearance of the cytoplasm around the former subsarcolemmal nuclei. It may be produced by the nuclei and excreted, as in the process believed to take place in the formation of the Nissl substance or even in the production of the plasmatic substance or some of its constituents, which surround the normal subsarcolemmal nuclei. It also may be that the cytoplasm, which appears newly formed, is an external material, attracted to and

modified by the subsarcolemmal nuclei as soon as the latter have been freed from their intrafibrous position.

To me it seems more probable that the basophilic cytoplasm in question corresponds to the muscular endoplasm. "Endoplasm" is the name given to that component of the sarcoplasm which under normal conditions surrounds the subsarcolemmal nuclei. Its position, mainly around the poles of the nuclei, has already suggested a comparison with

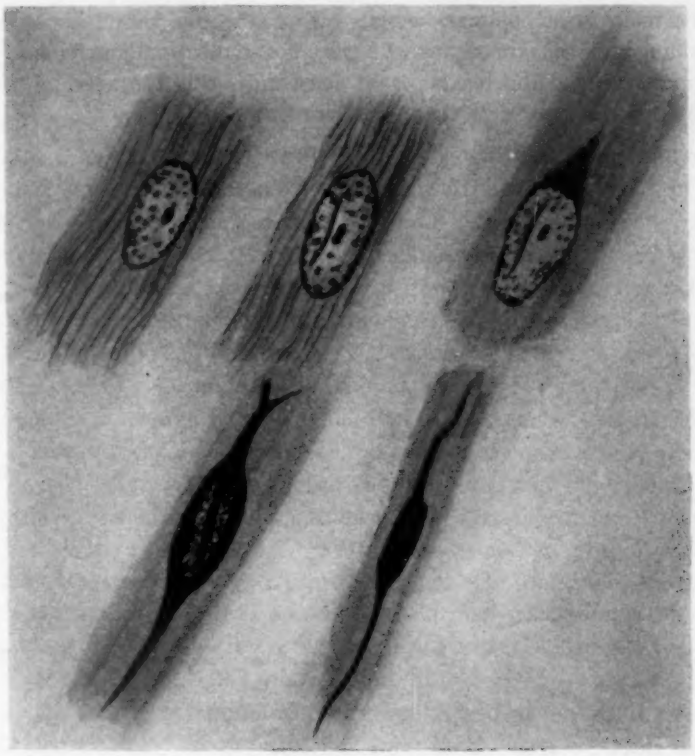

Fig. 2.—The progressive metaplastic stages of subsarcolemmal nuclei (half schematic).

the "so-called cells" in the connective tissue (Häggqvist [7]). But even before then, the subsarcolemmal nuclei, together with the surrounding cytoplasm, had been considered by Schultze [6] to be veritable single cells and were called muscle corpuscles. If that endoplasm is not destroyed during the process of degeneration and metaplasia, it is possible that it remains attached to the nucleus. At first it is not distinctly visible, but some time after the muscle fiber or its juxtanuclear part has ceased to exist, it changes in form and character, becoming basophilic and clearly discernible.

SUMMARY

Some aspects of muscle atrophy, degeneration and metaplasia have been studied. It is confirmed that the nature and extent of these processes depend not only on the duration of denervation but also on the species and type of muscle involved.

A new explanation is offered for the numerical increase of the subsarcolemmal nuclei in the atrophic process, by the decrease in tension inside the fiber, due to loss of cell material.

The metaplasia of muscle fibrils into connective tissue is confirmed. The change in the appearance of the subsarcolemmal nuclei and their development into fibroblasts or at least into cell elements of similar morphologic characteristics are described.

ARTERIOSCLEROSIS OBLITERANS

A STUDY OF THE LESIONS IN OCCLUDING PERIPHERAL SCLEROSIS, WITH A NOTE ON MÖNCKEBERG'S SCLEROSIS

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This paper is the third of a series on changes in the peripheral arteries. The first two papers [1] dealt with changes in the radial and the tibial arteries at various age periods. The specimens for those studies were taken in random cases but did not include any from patients in whom there were symptoms or other evidence of peripheral vascular disease. The present paper concerns the lesions in arteriosclerosis obliterans as represented in occluding peripheral vascular disease other than thromboangiitis obliterans. This means clinically, in terms of our material, cases of so-called senile and diabetic gangrene.

The literature on the clinical aspects of peripheral vascular disease is abundant, but detailed studies of the arteries themselves are relatively few. Bunge [2] thoroughly reviewed the literature and reported 15 cases of his own, divided into 5 of spontaneous gangrene, 5 of senile gangrene and 5 of diabetic gangrene. The spontaneous gangrene which he described was evidently the Winiwarter type. He found that the arterial occlusions in these 15 cases were due to intimal collagenous thickening culminating in thrombosis of the narrowed lumen. Only occasionally was there atheroma. Wartburg [3] and Manteuffel [4] wrote of marked intimal thickening in spontaneous gangrene and arteriosclerosis in the lower extremities but did not go into much detail. Mönckeberg [5] described medial calcification of the arteries of the extremities in 1903 and discussed its relation to atherosclerosis in 1914. Borchardt [6] cited

From the pathological laboratories of the Hahnemann Hospital and the Philadelphia General Hospital.

1. Sappington, S. W., and Cook, H. S.: Am. J. M. Sc. **192**:822, 1936. Sappington, S. W., and Horneff, J. A.: ibid. **201**:862, 1941.
2. Bunge: Arch. f. klin. Chir. **63**:467, 1901.
3. Wartburg, O.: Beitr. z. klin. Chir. **35**:624, 1902.
4. von Manteuffel, W. Z.: Mitt. a. d. Grenzgeb. d. Med. u. Chir. **10**:343, 1902.
5. Mönckeberg, J. G.: Virchows Arch. f. path. Anat. **171**:141, 1903; **216**:408, 1914.
6. Borchardt, H.: Virchows Arch. f. path. Anat. **259**:373, 1926.

26 cases of spontaneous gangrene but confined his discussion largely to the formation of new vessels, including new media, within the old stenosed vessels in 4 cases. In another paper [7] he wrote on the medial changes in the arteries in spontaneous gangrene. Bell [8] presented a complete review of the literature on the various aspects of arteriosclerosis of the extremities, and Ophüls,[9] writing on the genesis of arteriosclerosis, briefly discussed senile and diabetic gangrene of the lower extremities. Warren [10] also described arterial changes in gangrene of the lower extremities. Hines and Barker,[11] in an article under the newer title "Arteriosclerosis Obliterans," suggested discarding the terms "senile gangrene" and "diabetic gangrene" as representing a terminal process which in no way explains the underlying pathologic lesion. Their paper presents a clinical study of 280 cases of arteriosclerosis obliterans with a pathologic report on the arteries obtained from 32 amputated legs. They found as lesions atheromatous intimal plaques, degenerative changes in the media and thrombosis and stated that "actual obstruction of the artery is begun by the atheroma and completed by the thrombus" and further that "in arteriosclerosis obliterans, calcification and other forms of degeneration of the medial coat have no clinical significance in themselves, inasmuch as they do not cause significant interference with the flow of the blood."

MATERIAL AND METHODS

The material for this study consisted of 44 legs amputated under the diagnosis of arteriosclerotic or diabetic gangrene. For complete comparison, only those amputated above the knee were included. The arteries from these legs served as the basis for the study, though the extent of the gangrenous process and other significant external features of each case were recorded graphically. These arteries were obtained from the legs of 44 hospital patients, 22 males and 22 females. There were 31 white patients, 10 Negro patients and 3 for whom there was no record of race. There were 30 diabetic and 12 nondiabetic patients and 2 concerning whom there was no information on this point. The predominance of diabetic patients is probably attributable to the fact that a large diabetic clinic was the source. The average age was 67.4 years. There were 10 patients between 49 and 60, 16 between 61 and 70 and 17 above 70 years.

7. Borchardt, H.: Virchows Arch. f. path. Anat. **259**:521, 1926.

8. Bell, E. T.: Arteriosclerosis of the Abdominal Viscera and Extremities, in Cowdry, E. V.: Arteriosclerosis: A Survey of the Problem, New York, The Macmillan Company, 1933.

9. Ophüls, W.: The Pathogenesis of Arteriosclerosis, in Cowdry, E. V.: Arteriosclerosis: A Survey of the Problem, New York, The Macmillan Company, 1933.

10. Warren, S.: The Pathology of Diabetes Mellitus, ed. 2, Philadelphia, Lea & Febiger, 1938, pp. 121-142.

11. Hines, E. A., Jr., and Barker, N. W.: Am. J. M. Sc. **200**:717, 1940.

Within a few days after amputation, the main arterial tree was dissected out in one piece. This tree included the distal portion of the femoral artery, the popliteal, the anterior tibial and the proximal portion of the dorsalis pedis, the posterior tibial to its medial calcaneal branch, and the peroneal to beyond its major muscular branches. In a few instances the dissection was extended farther. A roentgenogram of the intact arterial tree was then made as a means

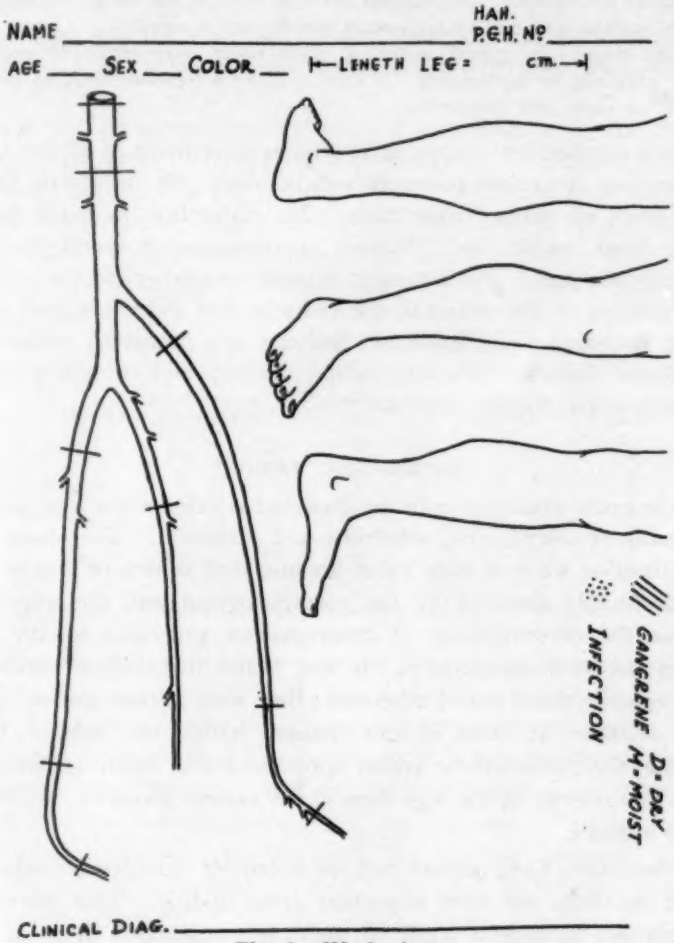

Fig. 1.—Work sheet.

of determining the location and the amount of calcification. After this the arteries were studied grossly by transections made at about every centimeter along their entire length, and notations were made of occlusions and other features. Segments were then chosen from certain regular locations for microscopic study: from the femoral artery just below the line of amputation; from the middle of the popliteal artery; from the anterior tibial artery high up and low down; from the dorsalis pedis about 2 cm. from its origin; from the posterior tibial artery below the branching of the peroneal (high), above the malleolus (low)

and just before the branching of the calcaneal artery beneath the malleolus (lower), and from the first portion of the peroneal artery. Figure 1 shows the work sheet on which the individual record was made, and on it are indicated the sites of the routine sections. In addition, other levels exhibiting interesting gross features were occasionally sectioned, and in a number of instances longitudinal sections were also made and studied.

Adjacent microscopic cross sections cut from each of the chosen segments were routinely stained with hematoxylin-eosin and by the Weigert-Van Gieson method for elastic tissue. In special instances, other stains were employed and frozen sections were cut for fat staining. A total of about a thousand sections were thus available for study and comparison.

While detailed and comparative studies were made of all the arteries in these cases, extended comment will be made only on certain features which seem of major importance. No distinction is made between arteries from "senile" and "diabetic" persons, as we found the lesions essentially the same. No comment is made on changes in the adventitia of the arteries or alterations in the veins as they did not appear of controlling importance. Significant findings are discussed under: (*a*) macroscopic lesions; (*b*) microscopic intimal and occluding lesions; (*c*) microscopic medial lesions.

MACROSCOPIC LESIONS

In the gross examination of the dissected arteries of the legs, notations were made of calcification, atheroma and occlusion. The observations on calcification were of little value because that substance was so much more accurately detected by the roentgenogram and the microscope. Likewise the determination of atheromatous processes by the naked eye was not very satisfactory. It was found that yellow patches did not necessarily mean mural atheroma; they were shown microscopically to be indicative at times of clot changes within the lumen. It was noted that the characteristic yellow appearance was much less frequently seen in the arteries of the legs than in the vessels above or, usually, the visceral arteries.

Occlusions.—The presence and the extent of vascular occlusion constituted decidedly the most important gross finding. This information was obtained by careful study of multiple transections made at about every centimeter along the entire length of each vessel. A vessel was not considered occluded when blocked by a red clot; occluded vessels were those which appeared entirely or practically closed by organized tissue. The entire length of the popliteal, the posterior tibial, the anterior tibial, the major proximal portion of the peroneal and the proximal end of the dorsalis pedis artery was thus inspected. Twenty-two arterial trees were satisfactory for examination in this respect. The findings as to the presence and the amount of occlusion agreed

very well with the microscopic data and, we believe, are approximately accurate. As far as errors are concerned, the microscopic control showed that most mistakes were made in the gross finding of closure of the dorsalis pedis artery. This was explained by the relatively greater thickness of the wall of this vessel, which along with minor changes made it appear closed. The mistakes in regard to the other vessels were few and were fairly well balanced by the occlusions detected microscopically in vessels where the gross appraisal had overlooked them.

In these 22 arterial trees, the surprising findings were that the lumen was occluded in about 13 per cent of the entire length of the popliteal artery, 50 per cent of the posterior tibial, 49 per cent of the anterior tibial, 33 per cent of the peroneal and 27 per cent of the dorsalis pedis artery. The average amount of occlusion of the posterior tibial, anterior tibial and peroneal arteries taken together was 44 per cent of their

TABLE I.—*Combinations of Vessels Occluded*

Popliteal	Anterior Tibial, or Dorsalis Pedis	Posterior Tibial	Peroneal	Cases
..	+	+	+	7
..	+	+	..	5
+	+	+	+	3
+	+	+	..	2
+	..	+	..	2
..	+	..	+	1
..	+	..	..	1
..	..	+	..	1
				22

entire length. The occlusions were not always continuous, and in some cases the figures are made from the sum of several blocked segments. But in a number of instances the vessels appeared occluded in from 90 to 100 per cent of their length, and even when the blocked portions were interrupted by clear passages, the percentage of the entire length involved by the individual occlusion was impressive. Such an amount of blocking contrasts strongly with the average coronary zones of occlusion which, according to Schlesinger and Zoll,[12] are mostly less than 5 mm. in length.

The combinations of vessels occluded are seen in table 1. The dorsalis pedis artery was occluded only once when the anterior tibial was not obstructed but was occluded six times when the anterior tibial was closed. The popliteal artery alone never contained an occluding lesion. In fact, it was shown that in the majority of cases popliteal blocks were secondary to those below. Complete occlusion at some point of all the main arteries of a level either in the popliteal or the lower leg region occurred in 14 of the 22 cases.

12. Schlesinger, M. J., and Zoll, P. M.: Arch. Path. **32**:178, 1941.

These findings as a whole indicate that the occluding lesions are widespread and that a remarkable percentage length of vessels is blocked before gangrene develops. One might say that in cases of arteriosclerosis gangrene is not necessarily imminent if either the anterior tibial or the posterior tibial pulsation can be felt along with the popliteal beat, since gangrene occurred in only 3 of 22 such cases.

MICROSCOPIC OCCLUDING AND INTIMAL LESIONS

Incidence of Occlusion.—The incidence of occlusion is shown in table 2 and represents the microscopic changes at the scheduled levels already indicated as well as those in unscheduled longitudinal sections. Occlusion was present in about 60 per cent of the posterior tibial sections at each level, about 40 per cent of the anterior tibial sections at each level and about 30 per cent of the peroneal arteries. It was less frequent in the

TABLE 2.—*Closure and Atheromatous Involvement of Arteries as Determined from Microscopic Sections of Scheduled Levels* and from Unscheduled Longitudinal Sections*

	Low Femoral	Popliteal	Peroneal	High Posterior Tibial	Low Posterior Tibial	Lower Posterior Tibial	High Anterior Tibial	Low Anterior Tibial	Dorsalis Pedis
Percentage of vessels closed	3	15	30	60	62	61	43	43	16
Percentage of vessels atheromatous	70	70	26	21	12	23	34	24	2

* See second paragraph under "Material and Methods" for scheduled levels.

dorsalis pedis sections, being observed in a little more than 16 per cent of the 44 cases, and this incidence lessened as the vessel was followed into the foot in the few cases so examined. The incidence of occlusion for the lower femoral arteries is about 3 per cent, and that for the popliteal arteries, about 15 per cent. The average number of vessels occluded per case was 2.3. The average number of occlusions per case at the scheduled levels was 3.5. In 1 case there was no occlusion in the vessels at the levels examined; in 1 case there were 8 occlusions; the majority showed 3 to 5 occlusions.

Nature of the Occluding Lesions.—The occlusions in different vessels varied considerably as may be seen in the figures, although as a whole they may be regarded as sequential. The earliest obstructions were obviously clots, and these were seen as collections of red cells, first without and then with fibrin threads (fig. 4 *A*). Following this, the mass sometimes became partially or completely hyalinized. The hyaline clot later occasionally became frankly atheromatous, with necrotic debris, including calcium and cholesterol crystals (fig. 2 *A*), a change noted

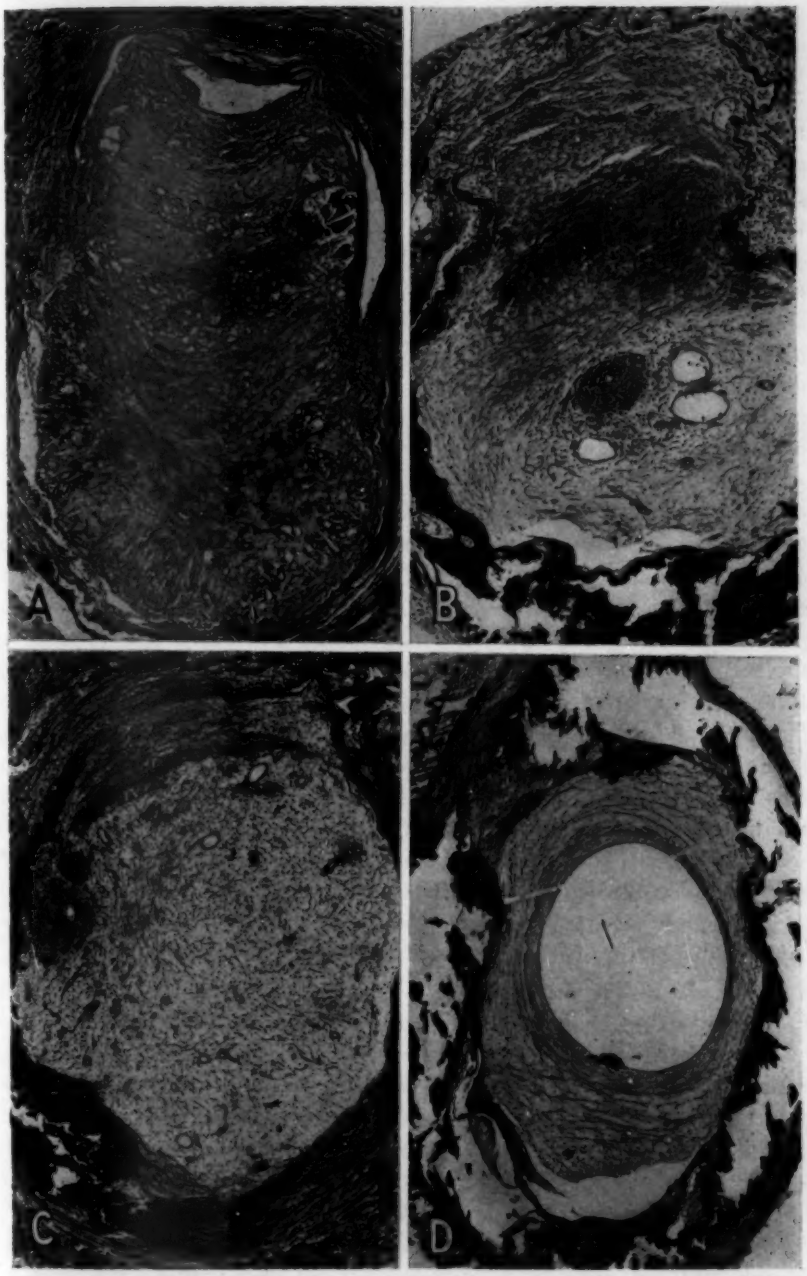

Fig. 2.—*A*, high cross section of a posterior tibial artery closed by a clot undergoing hyaline and atheromatous degeneration and secondary calcification. *B*, high cross section of a posterior tibial artery showing occlusion by delicate, partially hyalinized connective tissue, and some canalization. *C*, high cross section of an anterior tibial artery showing typical delicate connective tissue closure. *D*, high cross section of a peroneal artery showing partial closure by an organizing annular clot.

in hemorrhagic areas by Winternitz and associates.[13] More common was the evidence of beginning organization in the form of fibroblasts and delicate fibers among the red cells, together with more or less canalization (fig. 2 *B*). Transition stages were not always traceable to the next stage, which appeared to be the complete occlusion of the lumen by very delicate connective tissue fibers (figs. 2 *C* and 5 *C*), a picture quite similar to those labeled obliterative endarteritis or regenerative intimal thickening of Jores in textbooks. This is the first stage which we classed as a truly occlusive lesion. In some cases, the occlusion was not complete, and the tissue immediately about the remaining small or tiny lumen appeared condensed with closely concentric connective tissue fibers (fig. 2 *D*). Such developments occasionally progressed to the formation of a secondary muscular (medial) wall (fig. 3 *A*). As this was not infrequently associated with degenerative and necrotic changes in the deeper delicate connective tissue, the progressive concentration immediately about the lumen was interpreted as due to better nutrition from the still flowing blood stream. A later stage of the same picture was seen in sections in which the periluminal tissue was densely fibrous, and the peripheral portion of the occlusion relatively acellularly fibrous if not so dense (fig. 3 *B*). In sections with late, complete occlusions, the entire mass was densely fibrous and more or less contracted. In some cases the obstructing tissue became partly or even entirely atheromatous (fig. 3 *D*). This was more usual in the early stages but occurred at times also in the late dense tissue; in both instances the process was not mural but rather secondary necrosis in an organizing or organized thrombus. Such changes were the exception and not the rule. All this suggests that the occluding lesions represent various stages and regressions of organizing obstructive clots.

Changes in the Intimal Coat.—The intima itself did not present nearly as marked changes as the media or the occluding processes of the lumen. There were the usual findings of the age period—split elastica with interwoven connective tissue or muscle—and at times nothing more than this though the lumen might be completely closed with the delicate or dense fibrous tissue of an organized clot. Again, there was seen in addition to the changes characteristic of the age period some fibrous thickening, usually slight (fig. 3 *C*). This was mostly annular, occasionally eccentric in disposition. Yet again, the fibrous thickening, though still not extreme in degree, showed degenerative calcific and necrotic changes in the deeper portions, which might be interpreted as atheromatous and were plainly so in some instances. The approximate percentages of lumen closure and of atheromatous involvement of the

13. Winternitz, M. C.; Thomas, R. M., and LeCompte, P. M.: The Biology of Arteriosclerosis, Springfield, Ill., Charles C. Thomas, Publisher, 1938.

intima in the various arteries are seen in table 2. It should be mentioned that we extended ourselves in indicating an intimal change as atheromatous, the slightest evidence of mural necrosis being considered as warranting this description though the change may actually have been simple necrosis from lack of nutrition. Excluding the lower femoral and popliteal arteries, we may state that well defined atheromatous plaques as seen in the larger and visceral arteries elsewhere in the body were an exception and that the extreme eccentric atheromatous narrowings common in the coronary arteries were never seen.

One must be hesitant in thus signifying the mural changes in the intima proper as moderate or slight inasmuch as the orthodox conception stresses intimal atheroma as the initiating lesion in occlusion. But in table 2 it will be noted that in the larger arteries, e. g., the femoral and the popliteal, the percentage of intimal atheromatous sections was high, 70, while the percentage showing closures was notably low. On the contrary, the percentage of vessels occluded among those of the class of the tibial arteries was high while the percentage of atheromatous arteries was relatively low. This discrepancy was most striking in sections at the low level of the posterior tibial artery, which showed 26 of 42 vessels completely occluded whereas only 2 of the occluded vessels exhibited intimal atheroma. In the peroneal artery group, 8 per cent—one of 13 occluded vessels—showed atheroma. And in the high posterior tibial group but 3 of 25 closed arteries were atheromatous. The discrepancy was less striking in the anterior tibial group, in which the average was 1 of 3 or 4 occluded vessels showing atheroma. Our findings, in short, were that neither intimal atheroma nor fibrous intimal thickening featured as an outstanding or frequent lesion in the arteries of the leg below the popliteal and that the large majority of occluded vessels were free of mural atheroma at the site of obstruction.

The marked medial calcific changes, discussed later, were of a nature to suggest an influence, direct or indirect, on the flow of the blood and hence a bearing on occlusion, but definite conclusions in this respect were unjustified with our evidence. Thus, while we found that 50 of 71 occluded arteries were calcified and 21 not calcified, we also noted that calcification was present in 45 and absent in 13 of 58 open vessels. That the heavy lime salt deposit established a rigidity of the wall seemed clear from the fact that occluded vessels free of lime showed in many instances marked contraction throwing the intima into folds resembling those seen in the bronchioles in sections of lung (fig. 3 *C*), while vessels ringed with lime and occluded failed to show this contraction, presumably held rigid by the calcium (figs. 3 *B* and 5 *C*). Incidentally, many of these calcium-free contracted occluded vessels bore a striking resemblance to the occluded vessels seen in thromboangiitis obliterans.

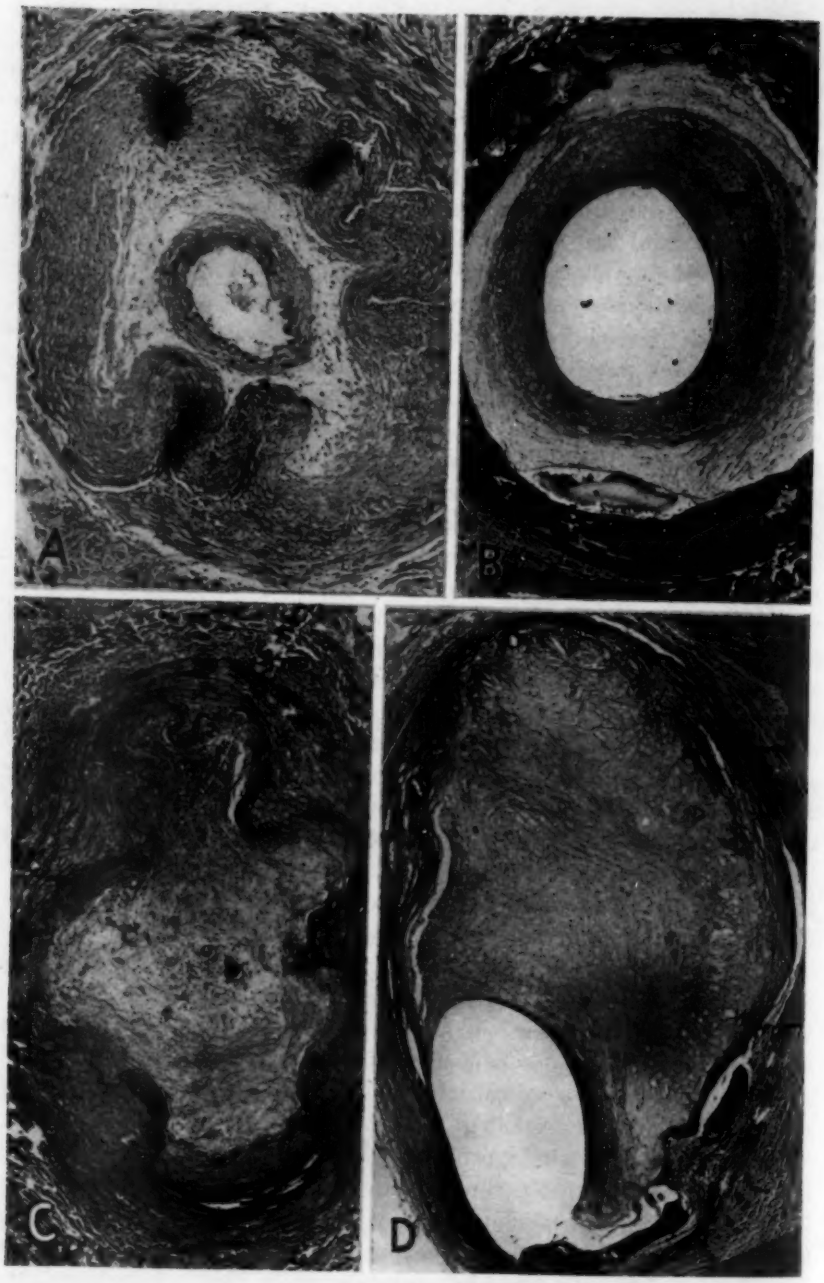

Figure 3

(See legend on opposite page)

If conditions in the intima or the media do not satisfactorily account for the occluding lesions of the arteries of the legs, how can these lesions be explained? We think the answer to this question is still wanting. With complete block of an arterial segment, it is to be expected that the flow of blood immediately above and below would be arrested and that in this static blood there would ensue first clotting and later organization. This would explain some occlusions of the lumens as simple secondary clottings in stilled blood (fig. 4 *A*) and would also account for the remarkable extent of the occlusions observed in some vessels. But the primary occluding lesion, which presumably should be marked by narrowing of the lumen from intimal change with final thrombosis, was hardly demonstrable. A theory more plausible to us is that the sum total of minor or moderate narrowings, irregularities of the lumen and mural rigidities from medial calcification and intimal thickening would induce eddies and slowing of the blood stream with a consequent definite tendency toward partial or complete thrombotic blocking. Canalizations in organizing thrombi partially filling the vascular lumen suggest this. In some vessels, too, we have noted, coating the endothelium, a thin annular layer of freshly formed delicate tissue together with some fairly fresh red cells, the whole picture suggesting a newly organized mural clot, formed possibly as the beginning result of the slowing of the blood stream. This conception has some support from the appearance in occluding lesions of several layers of thrombotic material of obviously different ages (fig. 4 *B* and *D*). In longitudinal sections, irregularities in the lumen may be plainly seen, and calcific medial bulgings and small intimal waves disturb the symmetry of the channel (fig. 5 *A*). The obstructive material varies, sometimes hyaline, again atheromatous and still again delicately or densely fibromatous, all suggestive of time variation in development (fig. 4 *C*). Particularly noted was a tendency toward longitudinal arrangement of delicate connective tissue strands as though some slight current effect had obtained during the formation of the material. It is noteworthy that in the coronary vessels mural thickening is often extreme and yet

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EXPLANATION OF FIGURE 3

A, middle cross section of a posterior tibial artery showing delicate connective tissue closure with formation of secondary media. Note infolding of the wall when the media is calcium free. *B*, very high cross section of a posterior tibial artery showing partial closure by an organizing clot of densely fibrous (central) and delicate (peripheral) connective tissue. *C*, low cross section of a posterior tibial artery showing closure of the lumen by delicate connective tissue. The intima shows slight uniform thickening. There is isolated calcification of the internal elastica, with mural infolding. *D*, high cross section of a peroneal artery showing a hyaline closing clot with marked secondary atheromatous degeneration (cholesterol clefts).

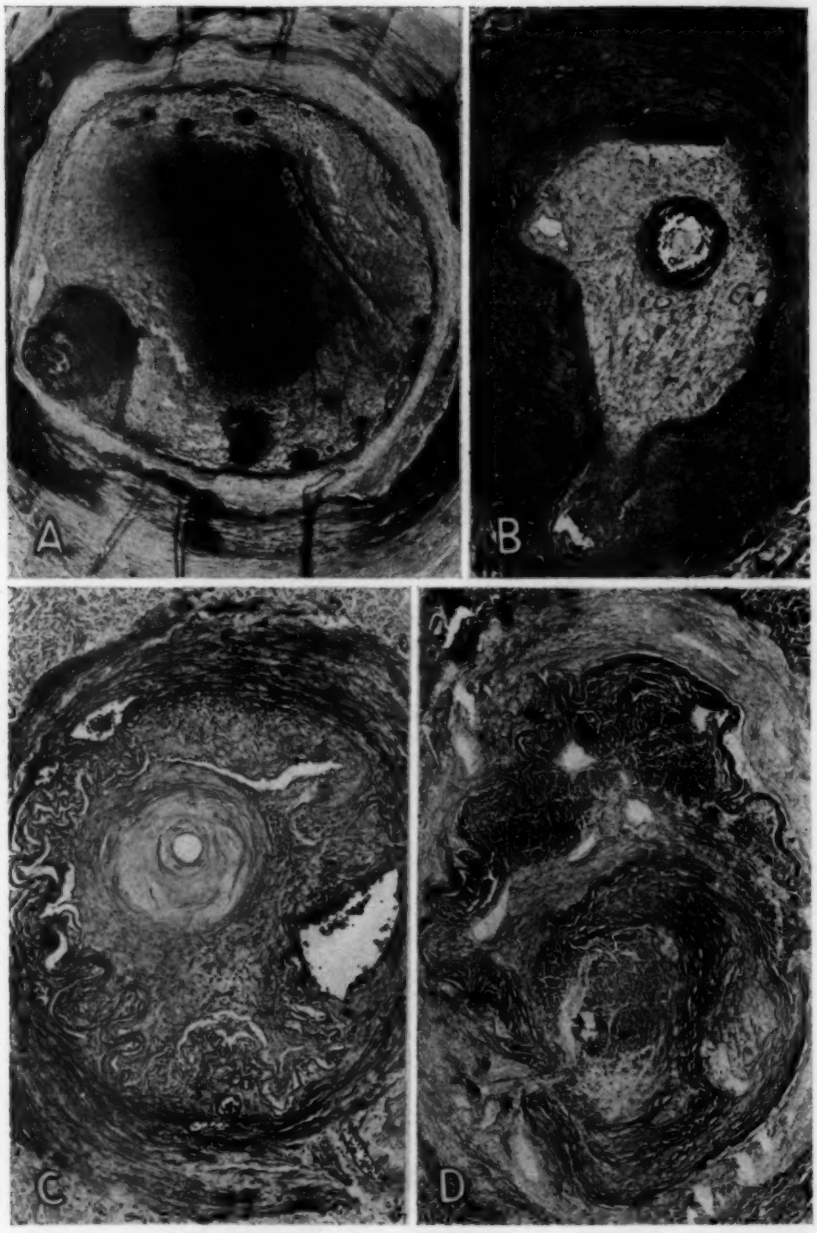

Fig. 4.—*A*, middle cross section of a posterior tibial artery with lumen closed by early clot, partially hyalinized. *B*, cross section of the terminal part of a posterior tibial artery showing multiple stage closure; the central closure is atheromatous and calcified. *C*, low cross section of a posterior tibial artery showing fibrous closure with central hyalinization. *D*, cross section in the highest part of an anterior tibial artery (Weigert-Van Gieson stain) illustrating closure in several distinct stages.

the tiny remaining lumen is clear of thrombotic closure, while in the arteries of the legs the comparatively large lumens are frequently and extensively blocked by organized clots without the initiating influence of atheromatous narrowings. That the luminal blockings are not necessarily at the same site as the mural lesions is quite understandable by the theory of slowed and altered blood flow, and in longitudinal sections of the arteries in our cases we have noted the presence of quite a hillock of atheroma without thrombotic result and in contrast complete and lengthy blocking without atheroma.

MICROSCOPIC MEDIAL LESIONS

General Observations.—The medial coat showed thinning and atrophy in about half of the vessels in our 44 cases. This process was slightly more marked in the posterior and anterior tibial arteries and on the whole was associated with the more marked intimal changes, which in turn seemed to account for the unevenness in thinning sometimes observable. It was least marked in the dorsalis pedis, where the media appeared a little thicker in about one third of the cases. In a small percentage of cases some of the vessels, confined largely to the posterior tibial group, were definitely contracted. Fibrosis, mentioned so frequently as a middle coat lesion, was not conspicuous in this series and was noted in only about one seventh of the vessels except in the femoral and popliteal groups, where it was present in approximately one third of the vessels.

There was some degree of cellular infiltration of the muscular coat in half of the posterior and anterior tibial arteries, in slightly less than half of the peroneal and popliteal arteries and in decidedly less than that proportion of the dorsalis pedis group. The degree was usually mild or moderate. In the few instances in which it was marked the intensity was unexplained by coincident conditions. The cells were mostly lymphocytes and histiocytes. Vascularity was a common concomitant condition. Vessels are negligible in the media of normal peripheral vessels when studied by ordinary methods but were demonstrable in about half of the vessels of this series. Both cellularity and vascularity were commonly absent or obscured in advanced calcification of the media but were associated phenomena of early retrogressive change and of ossification and were also related to intimal lesions. The cells in ossification were always part of a characteristically loose arrangement of fibrous connective tissue found invariably in association with bone formation and evidently furnishing the essential cells (fig. 5 *B*). Hemorrhage within the muscular coat was demonstrable in this series in only a few cases. It was not evident as a predecessor of calcification or ossification by the ordinary methods we employed.

Calcification.—The outstanding lesion of the media is calcification, though whether this is as important as it is common and obvious remains to be seen. How very common it is may be judged from the fact that it was demonstrated in 100 per cent of 38 cases in which the dissected arteries were examined by means of roentgenograms and in 98 per cent of the 44 cases in which they were examined microscopically. The incidence of involvement of the arteries at various levels is seen in table 3. Calcification was also very obvious in most cases, being usually present in abundance in large, deeply stained, sharply circumscribed foci of a significant configuration to be discussed later. Again it was seen in the form of fine grains of calcium between the muscle fibers, this finding being thought indicative of the beginning of the process. But these fine grains were frequently in proximity to massive accumulations, and in our 44 cases, in which the patients were practically all above 50 and mostly above 60 years of age, microscopic study showed

TABLE 3.—*Medial Calcification and Bone Formation in Cases of Arteriosclerosis Obliterans*

	Low Femoral	Popliteal	Peroneal	High Posterior Tibial	Low Posterior Tibial	Lower Posterior Tibial	High Anterior Tibial	Low Anterior Tibial	Dorsalis Pedis
Percentage of vessels showing calcification.....	90	72	80	76	66	48	82	75	66
Percentage of vessels showing bone formation..	16	19	19	43	33	23	45	31	12

plainly that the process of calcification as seen was in its late and, to our minds, final stage. In fact, it is safe to say that if a person lives long enough, there will certainly be found more or less medial calcification in the arteries of the lower extremities. The calcified foci were either centrally situated or nearer the inner aspect of the media. In the latter location, they were sometimes associated with the so-called "isolated calcification" of the internal elastica (fig. 3 *C*). Their presence at times occasioned an inward bulge encroaching on the lumen, an effect better observed in longitudinal sections (fig. 5 *A*). When extreme, it was apt to be mistaken for intimal involvement. Fractures of annular calcific bands were observed, most of these post mortem, some possibly ante mortem, a feature to which Gruber [14] gave considerable attention.

Bone Formation.—When ossification in sclerosed arteries was considered a rare or an occasional event, it did not arouse special interest, as it was well known that similar processes occurred elsewhere, as in the eye or the thyroid. But that it is more frequent than commonly

14. Gruber, G. B.: Virchows Arch. f. path. Anat. **275**:541, 1930.

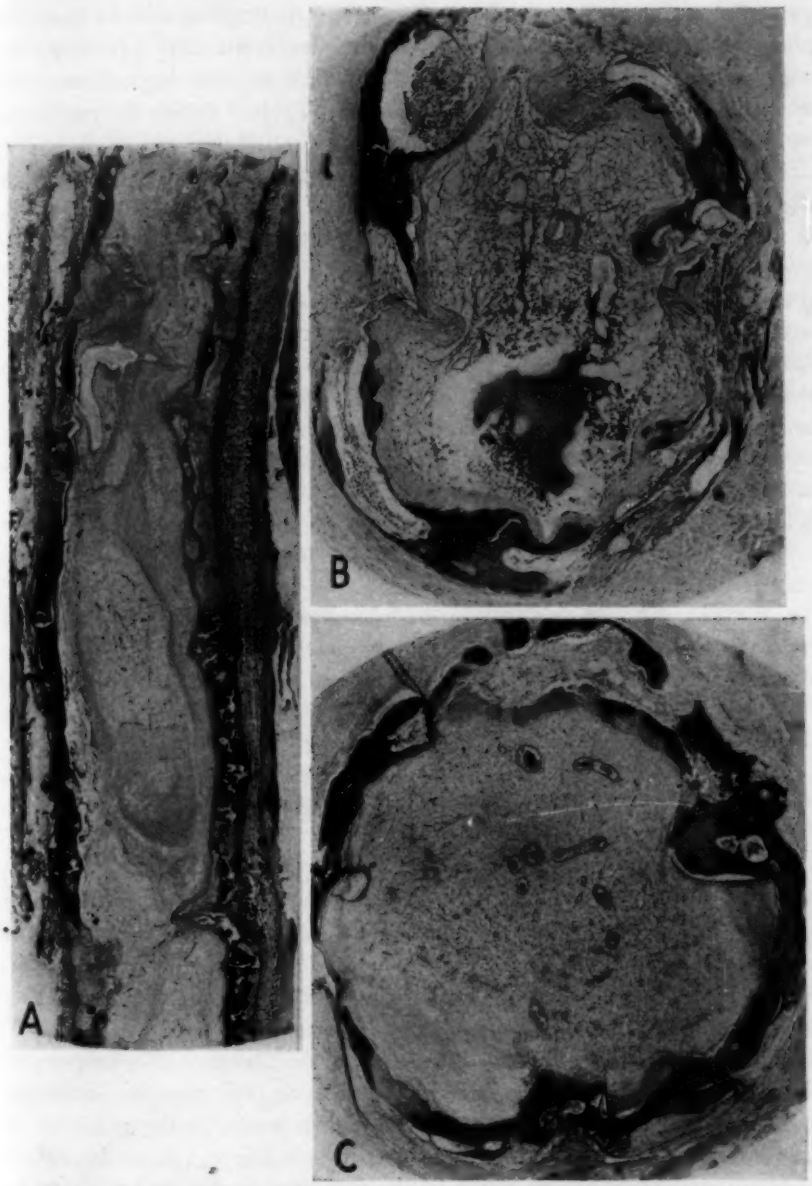

Fig. 5.—*A*, longitudinal section of a posterior tibial artery showing irregularities of channel and variations in the lumen-closing lesions. Note the sharp infolding of the wall. *B*, high cross section of a posterior tibial artery showing bone formation with secondary calcific changes. *C*, high cross section of a posterior tibial artery showing a complete bony ring with secondary calcific changes. Note closure of the lumen by delicate connective tissue without contraction of the wall.

thought was shown even by the early writers, and Mönckeberg [15] found bone formation in about 10 per cent of cases. If, however, it is actually as common as seems to be indicated by the findings in our 44 cases of peripheral sclerosis, namely, 70 per cent, its relation to preceding or concurrent lesions is probably of more than passing importance and the whole subject will bear reexamination. Table 3 shows the incidence of bone formation at various arterial levels in our series.

Bone formation in peripheral arteries occurs almost exclusively in the media and in unmistakable relation to medial calcification (fig. 5 *C*). It is this relation that we propose to reexamine. Pathologic calcification in general is thought of as depending on the deposit of lime salts in necrotic tissue, especially tissue rich in fats, and arterial calcification was considered no exception to this rule—this in spite of the fact that it has always been difficult to demonstrate preceding or concomitant degenerative processes in the media. To the casual observer, it often seemed a matter of wonder that such extensive calcific deposits were present without evident local cause, and many have commented on the absence of signs of reaction in the media about these calcified areas. Klotz [16] thought there was fatty degeneration with necrosis of the muscle fibers, accompanied by simultaneous laying down of calcium salts, which obscured the necrotic part of the tissue. When ossification was noted in or about the calcified foci, it was ascribed to metaplasia stimulated by the presence of lime salts and directed toward or resulting in resorption of these salts by the forming bone. Klotz [16] remarked, ". . . all authors have commented on this association of bone development with the preceding presence of calcareous deposit." In other words, the orthodox conception has been that ossification is a resorptive process developing after medial calcification has been established.

The study of our material inclines us to a theory which is the reverse of this, namely, that the attempt at bone formation in large part precedes and does not follow calcareous deposition in the media. This is the natural sequence, and our own and other evidence supports it. We may state our views as follows: The ossification-calcification process as a whole is an exceedingly chronic one and probably the result of multiple factors. The medial lesions are accessible—i. e., the amputated leg reaches the laboratory—only in the final or end stage of pathologic change, when the dominant and outstanding lesion is the presence of large calcium deposits, presumably in necrotic tissue. Bone formation is seen only occasionally because of the fact that conditions are observed at a late stage and also because an extensive study of many arteries

15. Mönckeberg, J. G.: Virchows Arch. f. path. Anat. **167**:191, 1902.
16. Klotz, O.: J. M. Research **34**:495, 1916.

from the same person is not usually made. Nevertheless, the basic relation of the bony and calcareous processes seems indisputable. Early some ill understood process eventuates in more or less medial necrosis not clearly detectable microscopically. This is followed by the appearance of characteristic cellular connective tissue which forms bone. The cells of this tissue may be the unused remaining mesenchymal cells referred to by Rohde.[17] This pathologic bone formation is not very vital and exhibits a marked chemotactic affinity for lime, which is promptly laid down in great excess and hastens the necrosis of the bone and the checking of the whole process. The rapidity of the deposition of lime and the volume of the deposits explain the relative dominance of calcification as contrasted with ossification, as well as the bony configuration of the calcific masses.

Our histologic observations suggest and support the foregoing views. Attention should first be called to the value of comparing sections stained with hematoxylin-eosin with those stained by the Weigert elastic tissue–Van Gieson method. With the Weigert method calcium shows as an amorphous light yellow substance, while bone takes a well defined plum color and retains its structural features. Arteries showing large deposits of calcium heavily stained by hematoxylin and no trace of bone may, by the Weigert method, show in the same location cleancut bone, which has been masked by the heavily stained calcium in the hematoxylin-stained section. In transverse sections of arteries ossification appears in part as isolated islands of bone or osteoid tissue and also as incomplete and complete rings encircling the vessel (fig. 5 *B* and *C*). In longitudinal sections it may appear as occasional islands of bone or as a more or less complete osseous layer paralleling the long axis of the artery. The process therefore tends to form enveloping sheets of bony material. In a careful study of these transverse and longitudinal sections, one sees the great variety of pictures which suggest to us the sequence of bone to calcium. Some fields show quite normal-looking bone, with no trace of calcium; others show all possible degrees of calcium deposition, indicated by the progressively deepening blue of the calcium up to a phase where all trace of bone is completely obliterated in the amorphous blue mass. These variations are so graded from clear bone to heavy calcium as to be unmistakably suggestive (fig. 6 *A*, *B* and *C*). Another common finding is small osseous foci at the end of a calcium patch or where there is a fracture of a limey ring. This was formerly thought, erroneously we believe, to be evidence of calcium resorption by bone. But in the Weigert mounts such sections may show the end patch of bone actually extending under the calcium mass; here, however, the bone is degenerated far beyond the relatively healthy state

17. Rohde, C.: Surg., Gynec. & Obst. **41**:740, 1925.

of the intact bone at the end of the patch. Another and significant picture is one in which the bone trabeculae take the curved or rounded configuration with development of lacunas or marrow spaces seen in any new bone production (fig. 6 *D*). The significance obtains from the fact that what appears to be bulky pure calcium deposit often presents exactly the same configuration. If now, these sections are stained by the Weigert method, some of them may show that underneath the obscuring calcium brought out by the hematoxylin stain there is really bone, and this bone is in a state of advancing degeneration rather than the healthy state which might support the theory of calcium resorption. Of course, in the large majority of instances bone is not demonstrable under these calcium masses for the simple reason that in the final stage in which the artery reaches the examiner there is complete necrosis with disappearance of bone.

There is some collateral evidence in favor of the view of bone formation preceding and not succeeding calcification. Recently, in a case of chronic osteomyelitis and also in a case of osteochondroma we have observed new bone formation in which there was succeeding degeneration with deposition of lime salts in large amounts so that the picture in certain fields was almost identical with that in isolated fields of the bone-calcium formation seen in the arterial wall. Klotz [16] noted that in experimental animals bone and cartilage may be induced to form in the arteries in the absence of preceding calcification. Harvey [18] painted the aorta of rabbits with silver nitrate or copper sulfate and produced in the media bone with marrow. On the other hand, Rohde [17] transplanted dead bone and various types of calcareous material to many parts of the body in experimental animals and was unable to find that metaplastic bone developed in a single instance. He said, ". . . the role of calcium salts in heterotopic bone formation in the soft parts, and especially in bone pathology, is of secondary importance." And further, "metaplastic bone building from the usual connective tissue . . . does not take place. Heterotopic bone formation in soft tissue is from the unused remaining mesenchyme cells."

SUMMARY

Forty-four cases of arteriosclerosis obliterans were studied by completely dissecting out the arterial trees from 44 amputated gangrenous legs. This paper is a report of the vascular lesions present, together with deductions from the findings.

Gross study showed an extraordinary amount of arterial occlusion, averaging 44 per cent of the entire length of the anterior tibial, posterior tibial and peroneal arteries and indicating the great degree of arterial obstruction preceding gangrene.

18. Harvey, W. H.: J. M. Research **17**:25, 1907.

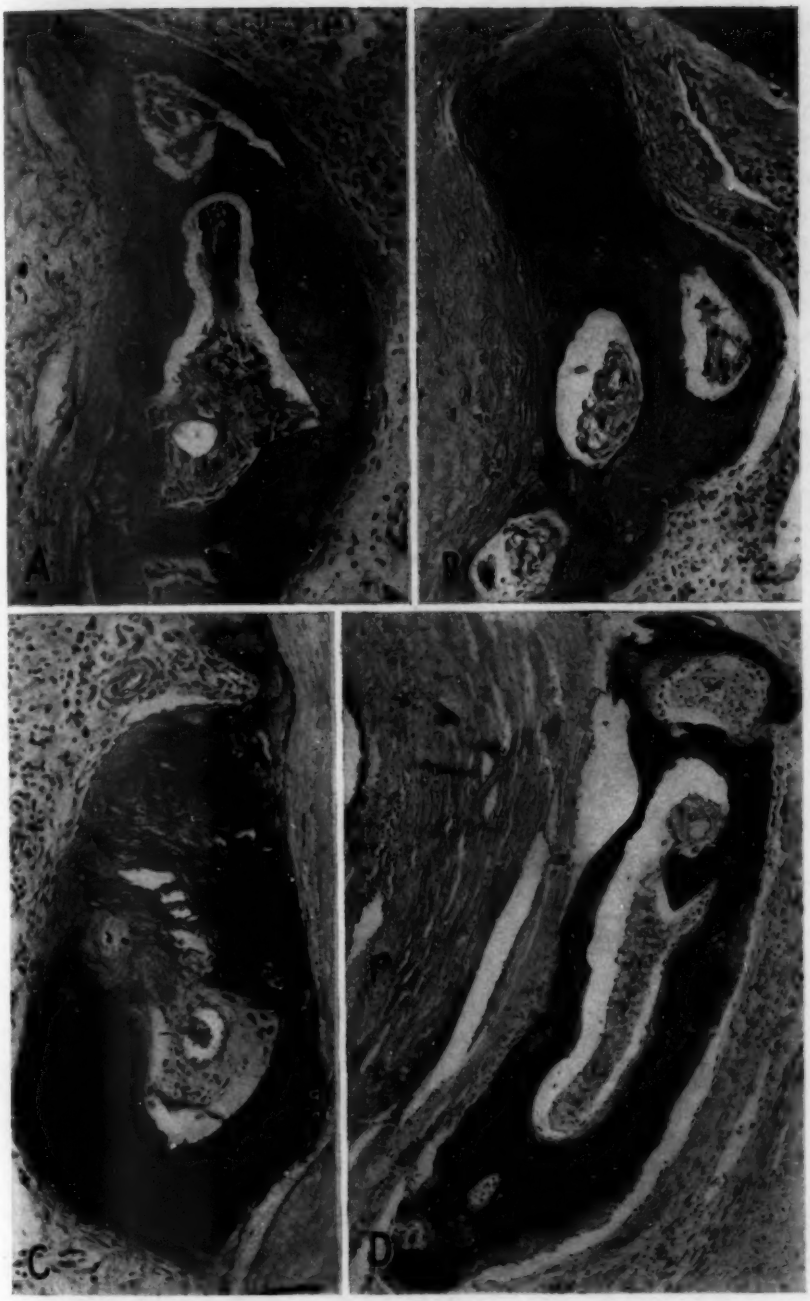

Fig. 6.—*A, B* and C, high cross sections of a posterior tibial artery, under high power magnification, revealing medial bone with progressive stages of secondary calcification. *D,* section from the lower part of a peroneal artery showing the typical configuration of medial bone formation with marrow spaces and osteoblasts. Note beginning secondary calcification.

Microscopic examination confirmed the gross estimate of arterial blockage. The average number of vessels occluded per case was 2.3. The occluding lesions apparently represented various stages and regressions of organizing obstructive clots. Atheroma was not a major or necessarily a participating feature. Vessels with the highest incidence of atheroma exhibited the lowest percentage of occlusions, while those with the highest incidence of closure presented the least degree of atheromatous involvement. It is suggested that abnormalities of the blood flow may be initiating factors in the formation of occluding clots.

The outstanding lesion of the media, calcification, was demonstrated in 100 per cent of 38 cases roentgenologically and in 98 per cent of 44 cases microscopically, but bone formation was also found in 70 per cent of the cases and a study of the obvious relationship between these two leads us to conclude that, contrary to the usual conception, bone formation precedes calcification in the media in arteries of the legs and probably accounts for the major part of the calcification found there.

HETEROTOPIC BRAIN TISSUE IN THE LUNGS OF TWO ANENCEPHALIC MONSTERS

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AND
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Heterotopic brain tissue in the lung is one of the rarest recorded congenital anomalies. In a fairly extensive review of the literature, we have been able to find reports of only 4 cases.

Because of their rarity and also because of their great interest from an embryogenetic point of view, we should like to add 2 of our own cases to this group.

REPORT OF CASES

CASE 1.—The body was that of a white stillborn girl weighing 3,450 Gm. and measuring 50.5 cm. in length. The characteristic faulty cranial development of anencephaly was present. The frontal bone was absent above the supraorbital ridge, the parietal bones were completely missing, and only the lowermost part of the squamous portion of the occipital bone had been formed. The base of the skull was flattened and was shortened in the anteroposterior diameter. The foramen magnum and the spinal canal were intact. A small mass of highly vascular abnormal brain tissue lay immediately above the foramen magnum and protruded through a coronal defect in the scalp.

The organs within the body cavities were normal except for marked hypoplasia of both adrenal glands and moderate hypoplasia of both lungs and the associated finding of several nonelevated white areas, measuring 1 to 2 mm. in diameter, on the lung surface. The majority of these nodules lay immediately beneath the pleura, but a few were found in the central portions of the lungs (fig. 1).

Histologically, the nodules were made up of tissue which is typical of that found normally in the brain. Large nerve cells, glial cells and fibers were included. Numerous capillary-like blood vessels were arranged in small clusters throughout the nodules. No alveoli were present, but a few bronchi were found in the periphery. The margins were irregular, and the abnormal tissue merged gradually with the lung parenchyma.

CASE 2.—The body was that of a 4 day old white boy weighing 2,945 Gm. and measuring 47.6 cm. in length. Anencephaly was noted, but the degree of abnormality was somewhat less marked than that in the preceding infant; the body was fairly normal with the exception of the head. The frontal bone was absent above the supraorbital ridge and the parietal bones were entirely missing. The spine was closed, the foramen magnum was present and the lower portion of the squamous part of the occipital bone had been formed. The spinal cord, medulla, pons and cerebellum were present but hypoplastic. The cerebral hemi-

From the Department of Obstetrics and Gynecology of the University of Chicago and the Chicago Lying-in Hospital.

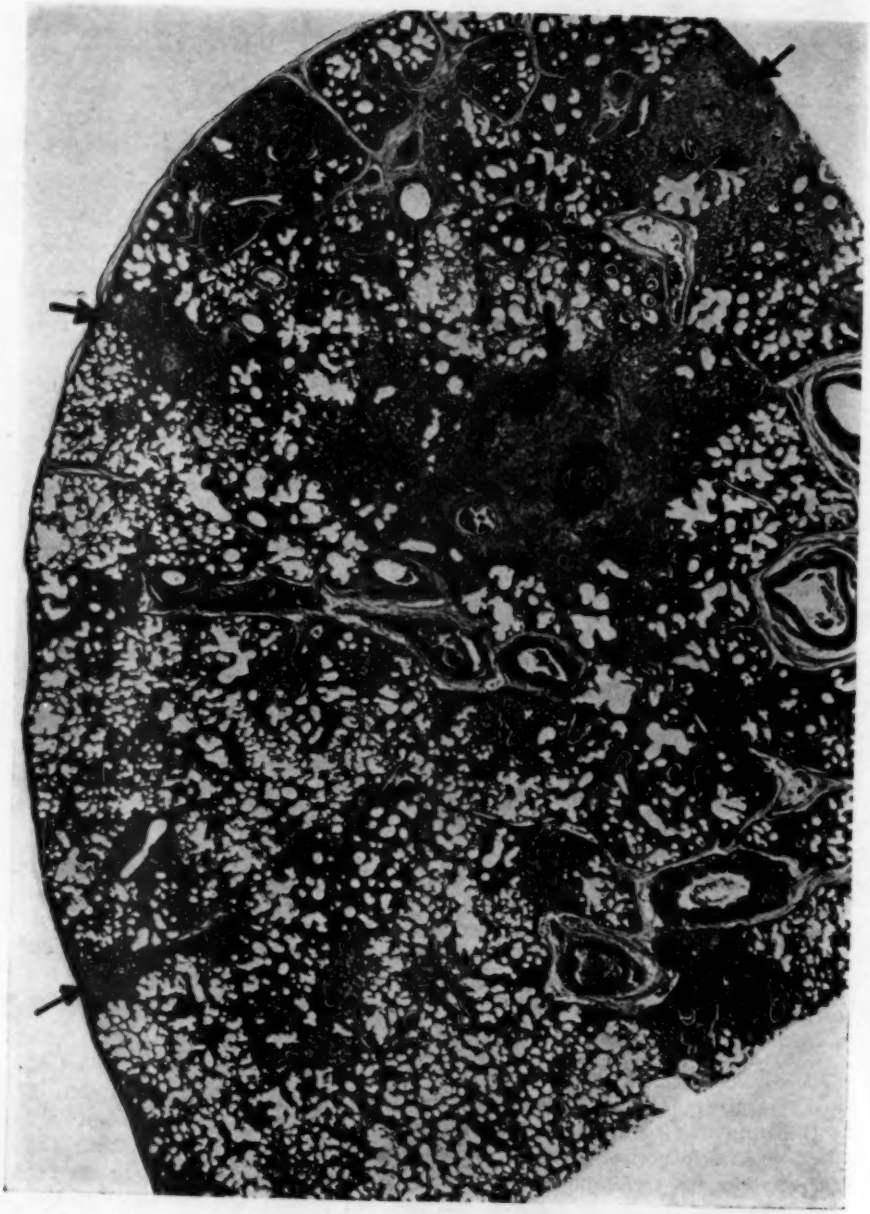

Fig. 1 (case 1).—The location of brain tissue in this portion of the lung is indicated by the arrows. Hematoxylin-eosin stain; × 8.

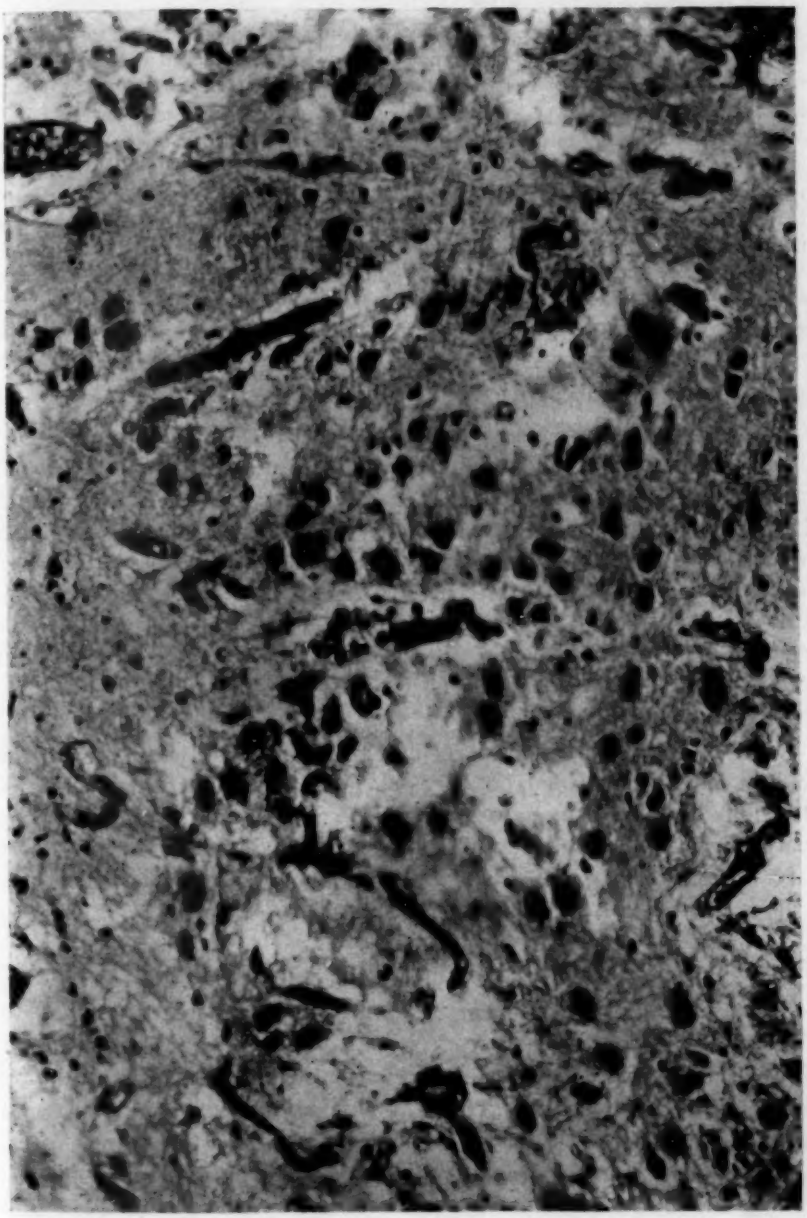

Fig. 2 (case 2).—The detailed structure of a portion of the brain tissue present in this lung is shown. Hematoxylin–eosin stain; × 225.

spheres were represented only by a small mass of vascular brain tissue which lay on the unenclosed base of the skull.

The body cavities and the viscera within them were normal except for extreme hypoplasia of the adrenal glands. The lungs were well aerated, and the only unusual feature was a white, firm, nonelevated area, 4 mm. in diameter, on the lateral surface of the lower lobe of the left lung.

Unfortunately, only one portion of each lung was saved for microscopic study. The right lung showed a moderate intra-alveolar infiltrate of leukocytes. The section of the left lung contained four areas composed of cells characteristic of brain tissue (fig. 2). There were a few bronchi and large blood vessels within these masses, and tortuous capillary-like vessels were present in clusters. The margins of the brain substance were irregular and extended outward into the pulmonary tissue. Two areas were present immediately beneath the pleura, and two were present in deeper-lying portions.

In both cases, sections of the lungs were stained by various methods, and the areas were definitely identified as histologically similar to brain tissue. Large nerve cells and characteristic glial cells were present. The small blood vessels within these regions had an arrangement not found in any normal tissue. This appearance suggested that peripheral growth had been inhibited and that the vessels had undergone proliferation within localized areas. It seems possible that these were capillaries which normally would form part of the alveolar walls but which in the absence of alveoli had remained in small compact groups. This appears to indicate that the ectopic tissue was present early in fetal development, before the normal invasion of the alveolar lining by capillaries takes place.

On the other hand, the irregular margins of the nodules with the existence of bronchi and large vessels within them is characteristic of the appearance produced when tumor cells become located in lung parenchyma and undergo local proliferation. It indicates the possibility of a peripheral growth of cells with local obliteration of alevoli and the partial persistence of bronchi and blood vessels.

COMMENT

Heterotopic brain tissue in the lungs would, from a review of the literature, appear to occur very infrequently. This impression may be due, however, to the relative infrequency with which necropsies have been made on fetuses and young infants in the past and the almost universal lack of routine histologic examination of the lungs. Furthermore, single cases are often deemed to be of insufficient interest for report, and for any one observer to see more than one example of this anomaly is most unusual.

During the past seven years, approximately 3,000 postmortem examinations on fetuses and infants dying within the first two weeks of life have been performed in the Chicago Lying-in Hospital. In all instances

the lungs, of macerated as well as of nonmacerated infants, have been examined histologically.

The incidence of heterotopic glial tissue in this autopsy material is 0.066 per cent. In a series of 1,700 congenitally malformed fetuses and infants on whom autopsies were made, these 2 infants were the only ones of their kind that were found.[1] This is an incidence among malformed infants of 0.12 per cent.

In 1908 Askanazy[2] reported multiple foci of glial tissue in the lung of a newly born boy who also exhibited microcephaly with associated herniation of both cerebral hemispheres through a defect in the skull. There was no tumor in the brain itself. Hückel[3] reported a similar heterotopic growth, classified as glioma, in the lung of a 4 year old child having a frontal meningocele.

In 1934 King[4] at autopsy removed a pulmonary tumor which bore a resemblance both grossly and microscopically to brain tissue. In this instance the patient was a 51 year old white man who had died of cerebral apoplexy. The tumor had been noted more than three years previously in a roentgenogram of the chest. It gave rise to no symptoms and showed no change in subsequent roentgenograms. No congenital malformations were noted.

Recently Gruenwald[5] found foci of glial tissue in both lungs of a 2 day old white girl having partial anencephaly associated with meningoceles extruding through two defects in the frontal bone.

Of great speculative interest is the manner in which such tumors arise. It has been suggested that the presence of brain tissue in the lungs of anencephalic monsters is the result of transportation of nerve cells to the lung from their normal location in the brain at the time when dissolution of brain substance is taking place. This theory presupposes that the varying degrees of anencephaly are due to actual destruction of previously formed brain tissue. In support of this belief are the relatively normal location and distribution of the cranial and spinal nerves in anencephaly; this and considerable other evidence suggests that in some instances, at least, anencephaly results from secondary destruction of brain tissue rather than from primary failure of closure of the neural canal.

Two theories have been advanced to explain the transportation of glial tissue to the lung: (1) that the tissue is carried through lymphatic or vascular channels and (2) that the tissue is aspirated. Three other possibilities may be hypothesized. First, there might be chance lodging

1. Unpublished data.
2. Askanazy, M.: Arb. a. d. Geb. d. path. anat. Inst. zu Tübingen **6**:433, 1908.
3. Hückel, R.: Verhandl. d. deutsch. path. Gesellsch. **24**:272, 1929.
4. King, W. I.: M. Bull. Vet. Admin. **15**:181, 1938.
5. Gruenwald, P.: Am. J. Path. **17**:879, 1941.

in the lung of glial fragments present in the amniotic fluid. Such a possibility does not seem reasonable because of the distance within the body which the cells would necessarily traverse before finally reaching the lung. Second, glial tissue might invade the lung by growth downward from the site of the lesion in the brain. If this were the process, one would expect to find occasional remnants of brain tissue along the course of spread. No such evidence has been found in any of the cases reported. Third, it is possible that mesenchyme may retain the ability to form nerve cells. If so, this tissue might then be incorporated in the lung during the lung buds' invasion of the mesenchyme. In view of more plausible evidence in support of the first theory, this seems an extremely remote possibility.

Anencephaly is produced very early in fetal life. Were the mechanism by which brain tissue arrived in the lung one of aspiration, implantation would have to occur after alveoli had been developed and after fetal respirations had been established. Such a stage is not reached until several months after the anencephaly has developed. It seems highly probable that the glial fragments would have degenerated before this time.

In the cases previously reported, the ectopic brain tissue was found only at the periphery of the lung. This has been considered evidence that the cells were blood borne. The presence of bronchi within the areas has been described as an invasion of well developed areas of foreign cells.

In our 2 cases the ectopic tissue was found in the more deeply lying portions as well as in the periphery of the lung. A few bronchi and large blood vessels were present within some of the areas, but the picture was identical with that produced when known neoplastic cells multiply in localized areas within the lung. The appearance suggests the invasion of the lung by ectopic cells rather than invasion of foreign tissue by growing pulmonary alveoli. It is most probable that both are growing simultaneously, that the heterotopic cells are deposited originally in the mesenchyme which is present between the developing bronchi and alveoli and that by their peripheral growth the development of local alveoli is inhibited and the bronchi are incorporated in the mass. That this tissue was not found in other heterotopic regions can be explained only by assuming that lung parenchyma is a more favorable medium for its growth.

Since in all but 1 of the cases the ectopic brain tissue was found in infants with gross abnormality of the brain, it is reasonable to suppose that in the majority of instances this abnormality predisposes to the existence of brain tissue in the lung. The case reported by King gives evidence, however, that destruction of brain tissue is not a necessary precursor to this condition and that cells probably may be trans-

ported from areas which are developing normally. Schultz [6] suggested that such a glioma developing in adult life arises from cells which have remained dormant in their ectopic position from early in fetal life until a time when some unknown factor initiates growth.

Although relatively few cases of glioma or ganglioneuroma in the newborn infant have been reported, many instances of extracranial location of such neoplasms in older persons have been recorded. Neuroblastoma is most common in the newborn period, and in a case recently reported by Potter and Parish [7] a common causation for abnormal and neoplastic development of various types of nerve cells was clearly shown.

We have also observed two other forms of ectopic tissue in the lungs of stillborn fetuses. In one fetus the lung harbored a small isolated mass of cells typical of adrenal cortex; in the other, a diffuse area containing large striated muscle fibers was present.

Both the adrenal and the muscle tissue must have arisen either from cells which had become detached from their point of origin and which had continued to grow in the new location or from cells which had arisen locally as a result of abnormal stimulation. The occurrence of such lesions indicates that cells characteristically found only in other parts of the body may occur in the lung in the absence of any known destruction or abnormality of the area from which they arise.

SUMMARY

Two cases of ectopic brain tissue in the lungs of anencephalic monsters are reported. A review of the literature reveals 3 similar cases, together with a case of such tissue in the lung of an adult who showed no malformation of the brain. It seems most probable that the nerve cells were carried to the lungs by the blood stream at the time when the lesions in the brain were developing.

6. Schultz, O. T., in Abt, I. A.: Pediatrics, Philadelphia, W. B. Saunders Company, 1926, vol. 8, p. 771.

7. Potter, E. L., and Parrish, J. M.: Am. J. Path. **18**:141, 1942.

TRANSPOSITION OF THE AORTA AND THE PULMONARY ARTERY

AN EMBRYOLOGIC STUDY OF ITS CAUSE

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Abnormalities of the heart involving transposition of the great vessels are so well recognized that further description of the condition is unnecessary. The normal relation between the pulmonary artery and the aorta as they arise from the right and the left ventricle, respectively, and the crossing of the pulmonary artery in front of the aorta diagonally to the left in a dextral spiral are so striking that any deviation from this pattern is obvious at a glance with the heart in situ. Accompanying or related abnormalities may include variation in the size of the vessels or of the ventricles, altered position of the cristae and the ledges, and failure of the usual septums to close or of the obliteration of the ductus arteriosus. Different combinations of these variables with transposition are recognized as of frequent occurrence, and some are so often associated that they have been dignified by special designations—the tetralogy of Fallot, Eisenmenger's complex—or have been classified as numbered types. Through all this group of anomalies the constant feature is the transposition of the great vessels.

Most writers see in the unusual position of the vessels evidence of detorsion of the heart tube in this region, an unwinding of the dextral spiral generally recognized as a part of normal development. Many consider such a statement as sufficient explanation of the anomaly; others seek in various ways a further reason for this local untwisting. Walmsley [1] suggested that the dextral rotation in the upper part of the tube has been completed normally, but that a sinistral spiral has developed in the lower portion, so that the two opposites cancel each other and parallel vessels result. Pernkopf and Wirtinger [2] postulated that the heart tube is rotated around its axis in two sections, each with its countertorsion, one in the region of the atrial canal and one in the region of the semilunar valves. The two are independent of each other, and in transposition the semilunar torsion fails to take place. Möncke-

From the Harvard Medical School.

1. Walmsley, T.: J. Anat. **45**:528, 1931.

2. Pernkopf, E., and Wirtinger, W.: Ztschr. f. Anat. u. Entwcklngsgesch. **100**:563, 1933.

berg [3] saw in the frequent occurrence of this anomaly in families and generations and in its frequent association with other bodily anomalies a possible underlying cause in the germ plasm. Spitzer,[4] whose philosophic ideas have been so aptly translated by Harris and Farber,[5] called on phylogeny for an explanation and suggested that the anomaly represents a reversion to the reptilian condition and the preservation of two aortas, one arising from each ventricle, yet with the further complication, not found in reptiles, that the aorta from the left ventricle becomes simultaneously obliterated. No cause for this atavistic reversion to a more primitive animal type is suggested. The condition in the heart described by Liebow and McFarland [6] as showing both reptilian aortas, in support of Spitzer's theory, can be better explained, it seems to me, as analogous to tracheoesophageal fistula with the stenosis of the lower part of the esophagus commonly found in that anomaly; in this heart, however, the added action of the counterpressures of systole and diastole would distort the upper and lower segments of the stenosed pulmonary artery, forcing the upper segment against the roof of the ventricle to simulate a third trunk. In none of the theories advanced heretofore have embryologic facts been critically considered.

From the point of view of embryology it seems probable that an anomaly so relatively frequent and so constant in its basic form will prove to be due to some slight and easily attainable variation from the normal development of the organ, occurring while that organ is in a plastic state, usually at a very early period. Since transposition is reported as rare among animals, the search for the variation in question was chiefly pursued among human embryos, and the Harvard Embryological Collection offered a goodly supply for the intensive study of the early development of the heart. This study and the critical review of many of the published descriptions of hearts in early embryonic stages of development supply the basis for the present paper.

Anatomic usage considers that the limits of the two ventricles of the adult heart are the two sets of cardiac valves, the atrioventricular mitral and tricuspid valves proximally and the two semilunar valves distally, in spite of the fact that sinusoids, which are the most characteristic feature of the ventricles, are not present everywhere between these limits, being absent from the conus or infundibulum of the right ventricle and from the less conspicuous analogous region of the left. In the early stages of heart development the semilunar valves are formed side by side on two spirally arranged channels within the common arterial trunk, and it occurred to me that their relative anteroposterior

3. Mönckeberg, J. G.: Herzmissbildungen: Ein Atlas Angeborener Herzfehler in Querschnitten, Jena, Gustav Fischer, 1912.

4. Spitzer, A.: Virchows Arch. f. path. Anat. **243**:81, 1923.

5. Harris, J. S., and Farber, S.: Arch. Path. **28**:427, 1939.

6. Liebow, A. A., and McFarland, W.: Arch. Path. **32**:356, 1941.

position might be altered by a simple change from one level to another along the spiral tube. The more distally the valves were placed, the less of the spiral arrangement would the distal part of the tube beyond the valves show, in other words, detorsion would be apparent. However, the available human embryos in which the semilunar valves were indicated or well developed, and many other embryos of pigs, rabbits and rats, showed a remarkable uniformity in the position of the valves, and thus precluded all hope that the explanation of the anomaly lay in that direction.

The shapes of the hearts of early embryos showed, on the other hand, a large measure of variation, as a study of the drawings of reconstructions culled from different publications (fig. 1) clearly shows. The basic steps in heart development can be readily followed. As is well known, the heart, which is a double-walled tube with the outer wall of epimyocardium and the inner wall of endothelium, grows faster than the pericardial cavity within which it is confined and is forced to bend. Further lengthening causes it to form a loop (fig. 1 *a*) to conserve space even further. The drawings represent the inner, more slender endothelial tube, as far as such reconstructions are available, since these often show the relations more clearly than the plumper exterior tube. The loop form results in a full spiral torsion of the free part of the tube between the two fixed ends, as can readily be proved by bending a rubber tube in the form of a closed loop. The bend in the heart is normally to the right, and results in a dextral spiral torsion along its length. This condition, so obvious in embryonic hearts and in the dextral torsion of the adult pulmonary artery around the aorta, has given rise to a fallacy often repeated by anatomists, to which I also subscribed in former papers, and perhaps calls for the interpolation of a few lines of clarification.

The fallacy lies in the theory that since the two ends of the heart tube are fixed at their exit from the pericardial cavity so that there is no possibility of rotation, there must be somewhere along the free tube a region of sinistral torsion to cancel the obvious dextral torsion already noticed. Pernkopf and Wirtinger's suggestion of two modes of torsion and detorsion has already been given, but these nodes have never been identified. Spitzer found the necessary detorsion in the shape of the valves of the sinus venosus, as "a glance at the Born model will demonstrate." Actually the sinistral or compensating torsion is seen in the shape of the loop itself, as can be recognized in figure 1 *a*. The whole tube makes one full sinistral turn in forming the loop; this it is that compensates the dextral spiral torsion, and further search for a region of detorsion is unnecessary.

The pictures of other heart tubes given in figure 1 show that the basic loop form may present individual variations. As the tube lengthens.

secondary bends appear, the tube becomes crumpled, folding back on itself in some regions until the round loop can scarcely be recognized. Expansions and constrictions mark off different segments as the

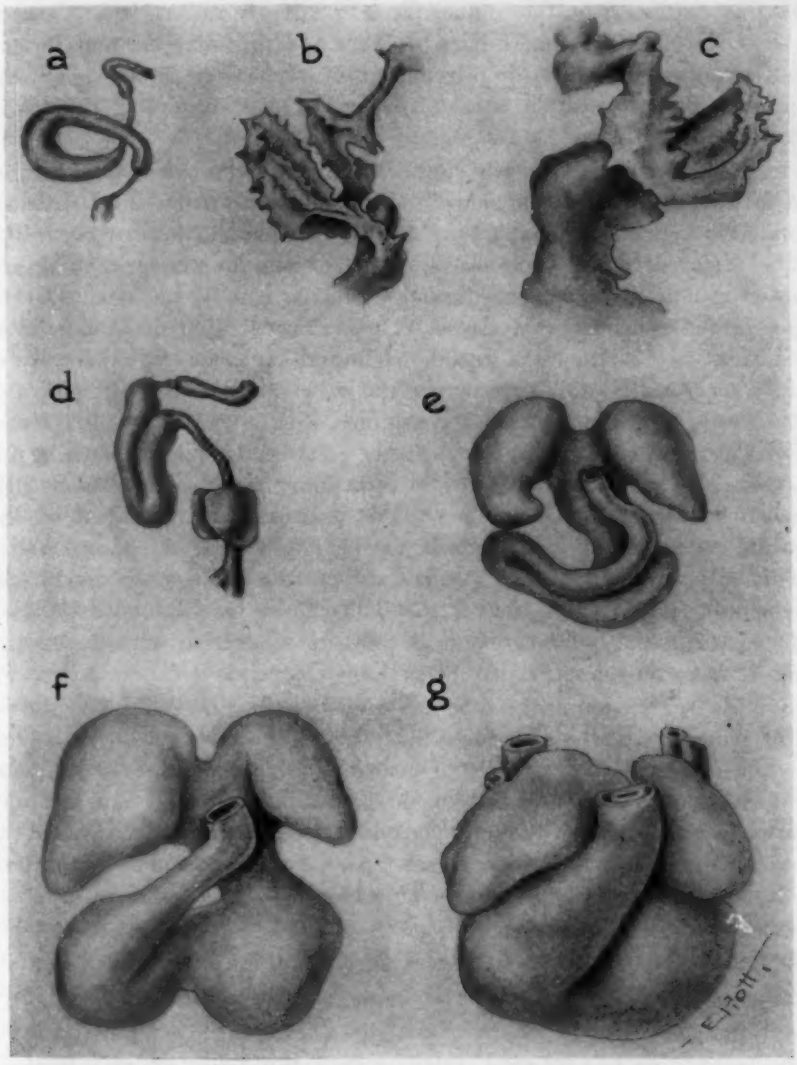

Fig. 1.—Copies of drawings of models of the endothelial hearts of young human embryos, to show the loop formation and later alterations in shape within the pericardial cavity. In the lateral views the venous end opens from below and the arterial end is above, the axis of the embryo being vertical. (*a*) 2.15 mm. from left side, His; (*b*) 17 somites, left side, Atwell; (*c*) 20 somites, right side, Davis; (*d*) 4.2 mm. left side, His; (*e*) 4.25 mm. ventral view, His; (*f*) 5 mm. ventral view, His; (*g*) 4.9 mm. ventral view of whole heart, Ingals (Arch. f. mikr. Anat. **70**:506, 1907). *b* and *c* show the subendothelial fibers mentioned in the text.

expanded atrium, the narrow atrioventricular canal, the ventricle, the bulb and the arterial trunk. These are well defined in an embryo of 4.25 mm. (fig. 1 *e*). The chief variations in shape are in the bulbar region and seem to be due to differences in the length of this segment. In this characteristic the human heart apparently differs from those of the other mammals that I have studied, and this may account for the infrequency of transposition reported in the lower animals, for the anomaly of transposition is found to depend on the shape of the bulbar curve at these early stages.

As development proceeds certain portions of the coiled tube become relatively fixed. The caudal wall of the common atrium fuses with the underlying primitive diaphragm, through which the lower veins pass. The atrioventricular canal becomes more solid by the growth of the anterior and posterior endocardial cushions, caused by local thickening of the subendothelial tissue. Their future fusion, leaving slits at the sides, divides the canal into the right and left atrioventricular valves; their mass makes this segment of the tube more solid and rigid, and the posterior cushion, by its continuity with the atrial wall, is also fixed in position. Beyond the canal the tube bends sharply to the right, so sharply that the canal seems to open into the dorsal wall of the left ventricle, rather than into its end, an appearance emphasized by the future expansion of the ventricle to the left beyond the mitral valve. This disposition fixes this segment of the tube. The only region still remaining relatively flexible is the bulbar limb of the loop. As His [7] expressed it, the bulbar portion, beyond its connection with the ventricle, is free "and describes from there on independent bendings."

This free section of the tube is variously named by different authors. For some it is the bulbus; for others, the right ventricle. It is common to consider that the ventricle in hearts such as those shown in figure 1 *d* and *f* comprises a descending and an ascending limb, and the surface furrow between them is called the interventricular groove; at other times the same authors may call the same indentation the bulboventricular groove. Both sets of terms are correct, but at different periods of development. Keith [8] spoke of the right ventricle as encroaching on the bulb, as though it were derived by an extension of the left ventricle, the bulb meanwhile becoming relatively and actually shorter until it is represented only by a small area close to the semilunar valves. He pointed to a transverse ridge occasionally found at this level as the lower border of the bulb in the adult heart. These ideas of the retrogression of the bulb and its replacement by an extension of the

7. His, W.: Anatomie menschlicher Embryonen, Leipzig, F. C. W. Vogel, 1885, pt. 3.

8. Keith, A.: Lancet **2**:359, 1909.

left ventricle to form the right ventricle must be revised; structures that are charactertistic of the bulbar region persist in the adult right ventricle, and the study of its development shows that the ventricle grows within the bulb instead of replacing it as it shrinks to smaller dimensions; the adult right ventricle comprises the major part of the embryonic bulb.

That bulbar structures persist in the ventricle has been tacitly accepted. As is well known, the separation of the aortic and pulmonary streams is accomplished by the growth and fusion of paired longitudinal ridges following a spiral course through trunk and bulb. As Tandler [9] showed, these are developed in three sections: the aorticopulmonary ridges in the trunk, and the distal and proximal pairs of bulbar ridges in the bulb. The semilunar valves develop, according to Langer,[10] from the distal ends of the proximal pair, so that the remainders of the proximal ridges lie entirely within the adult ventricle. Their fusion to separate the two streams begins above and is never completed below, The two ridges extend down to the point where the bulb turns sharply to the left to join the right end of the left ventricle, and here are situated on the left anterior and the right posterior wall of the tube. The left ridge (known as A) joins the side of the interventricular septum and continues further as the moderator band. The right posterior ridge (B) spreads out widely on the outer convex wall of the tube. Two small branches turn to the left to join the right wall of the atrioventricular canal, where they later become associated with the medial and lateral cusps of the tricuspid valve; the main broader portion continues downward laterally. This branch, with the free lower edge of the uncompleted septum and the upper part of ridge A together form the supraventricular crest of the adult ventricle. Thus the adult derivatives of the two proximal bulbar ridges lie within the right ventricle.

The older views on the formation of the ventricles are no longer tenable. It used to be taught that in certain expanded areas of the heart tube muscular trabeculae grew inward from the cortical layer, partially filling the cavity and carrying the endothelial layer before them in such a way as to leave the vascular sinusoids between them. Born [11] thought this trabecular structure or spongy substance of so little consequence that it was entirely left out of his well known models of heart development. Actually, growth is in the opposite direction (Greil [12]). The musculature of the embryonic ventricular and bulbar

9. Tandler, J.: The Development of the Heart, in Keibel, F., and Mall, F. P.: Manual of Human Embryology, Philadelphia, J. B. Lippincott Company, 1912, vol. 2, p. 534.

10. Langer, A.: Morphol. Jahrb. **22**:99, 1894.

11. Born, G.: Arch. f. mikr. Anat. **33**:284, 1889.

12. Greil, A.: Morphol. Jahrb. **31**:123, 1903.

regions develops as two separate sheets, more or less like the two sets of muscles in the intestinal wall, the muscularis proper and the muscularis mucosae, but with the difference that in the heart the inner, subendothelial layer is the heavier and the first to develop definite muscle fibrils (Tandler [9]). It is circular in disposition and at first lies at some distance from the endothelium, and the space between it and the cortical layer is filled by loose tissue. As has been shown in a former paper (Bremer [13]), the strands of premuscular tissue apparently separate slightly on the convex walls of the heart tube at points of sharp curvature, much as the strands of any binding material wound closely around a rubber tube would spread apart on the convex wall if the tube were sharply bent. Toward and into these gaps in the muscular coat endothelial sprouts make their way, like minute aneurysms, to spread and anastomose in the tissue below. The sprouts follow the pathway afforded by the delicate fibrils which Davis [14] described as the earliest signs of organization of the formerly structureless subendothelial tissue, occurring on the convex walls of ventricle and bulb (fig. 1 *c*). Similar fibrils are shown by Atwell [15] (fig. 1 *b*) and by Congdon [16] in a human embryo of 3 mm. with 22 pairs of somites. Soon the muscular strands follow between the sinusoids, growing peripherally to join the cortex. The free inner tips of the trabeculae, rather than the cortex, mark the true inner limit of the muscular heart wall.

The sharpest curvature for the left ventricle points forward, downward and to the left (fig. 1 *e*). The sinusoids therefore develop in these directions, causing by their growth the rounded pouch of the ventricle. The right side of the posterior wall has few if any sprouts growing from it, since it is concave instead of convex, and remains relatively smooth in the adult. At the other end of the interventricular canal the lower portion of the bulb forms a pouch caudally as it turns the acute angle from the horizontal canal to the ascending limb of the bulb. The curvature is greatest on the lower or caudal surface, and here sinusoids will appear. But, as in the left ventricle, the direction of the apex of the angle is not purely downward. The bulb points also laterally to the right, and forward or ventrally, as is seen in the model (fig. 1 *g*), where the bulb is shown as set somewhat on the anterior surface of the interventricular canal. The sinusoids, developing on the convex surface of this endothelial tube, will therefore form a ventricle bulging downward, to the right and forward; the posterior wall will be relatively smooth. The ventral interventricular septum represents the remaining portion of the inner layer of the musculature persisting as a ridge between the sinuosids of the two ventricles.

13. Bremer, J. L.: Am. J. Anat. **42**:307, 1928.
14. Davis, C. L.: Contrib. Embryol. **15**:3, 1923.
15. Atwell, W. J.: Contrib. Embryol. **21**:1, 1930.
16. Congdon, E. D.: Contrib. Embryol. **14**:47, 1922.

None of the hearts shown in figure 1, ranging up to 5 mm., is old enough to have developed sinusoidal sprouts in either ventricle. They are present, however, in the heart of the embryo of 6.7 mm. shown in figures 2 *a* and 3 *a*. Figure 2 is drawn from a reconstruction of serial sections and represents a longitudinal view of the bulbar portion split sagittally as if along the broken line in the small insert and looked at from the right side. The sinusoids are seen on the caudal and anterior or ventral walls of the bulb. This seems to be the usual or normal mammalian disposition if one may form any judgment from the few human embryos of similar age available to me for study and from a greater number of embryos of other mammals. In the chick

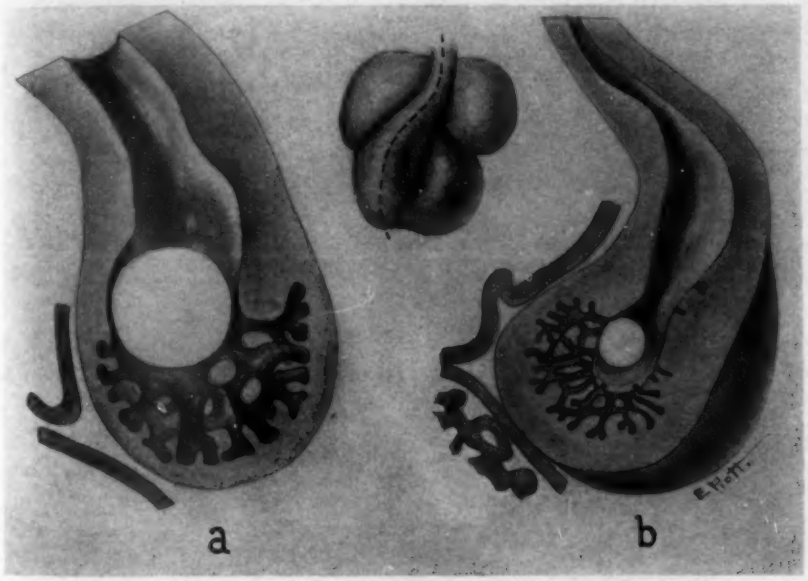

Fig. 2.—(*a*) Reconstruction of the bulbar portion of the heart of a human embryo of 6.7 mm. The specimen is represented as though cut sagittally along the broken line in the small inserted figure, viewed from the right side. The bulboventricular canal is indicated. The ventricular sinusoids sprout from the apex and from the ventral wall. The lower wall of the right atrium and the septum transversum are indicated dorsally. × 35.

(*b*) Similar view of a reconstruction of the bulbar portion of the heart of a human embryo of 6.3 mm. The apex of the bulb turns dorsally toward the septum transversum; the bulbar sinusoids sprout from the apex and the dorsal wall. The left ventricle projects ventrally beyond the bulb. × 35.

(Bremer [13]) the sharpest curvature of the bulb is at a higher level and the right ventricle is initiated in a more distal position. Figure 3 *a* is a photograph of the ventricles of this heart from a transverse section at the level of the interventricular canal. Again the sinusoids predom-

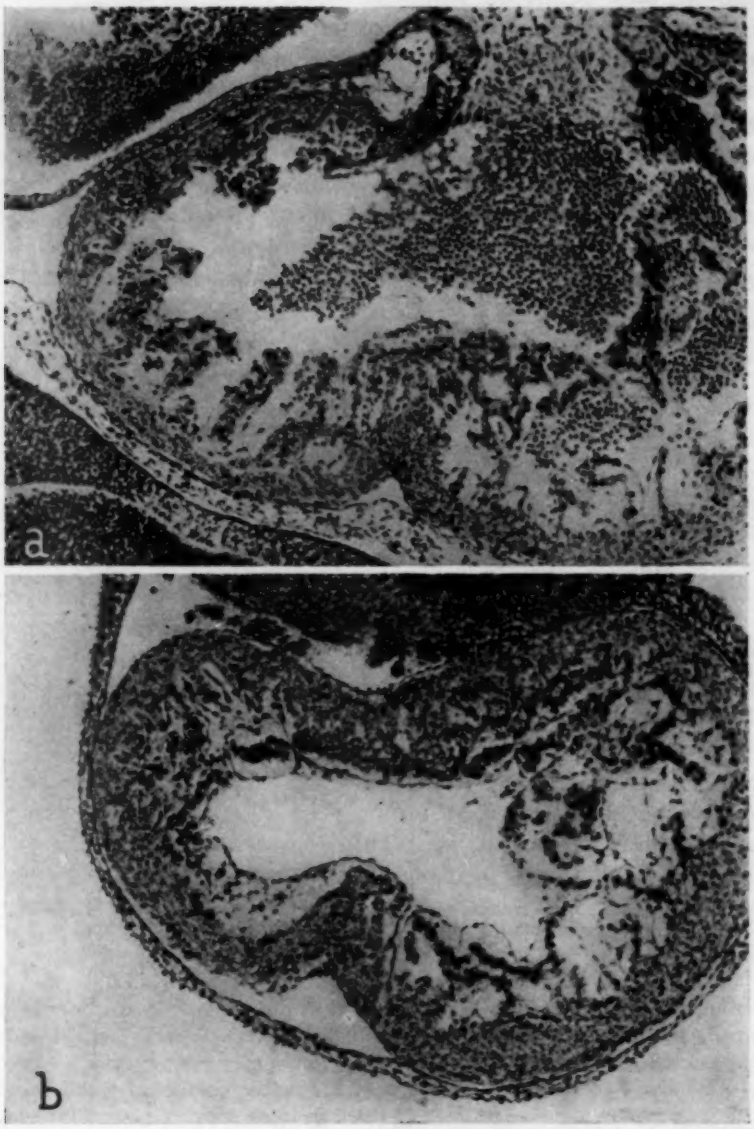

Fig. 3.—(*a*) Transverse section through the lower part of the bulb (at left in photograph), the bulboventricular canal and a portion of the left ventricle of a human embryo of 6.7 mm. (compare fig. 2 *a*). The deepest bulbar sinusoids project ventrally; the dorsal wall of the bulb rests against the lower wall of the right atrium. The flexed head shows in the lower corner. × 75.

(*b*) Similar section of a human embryo of 6.3 mm. (compare fig. 2 *b*). The bulbar sinusoids (at left in photograph) project dorsally; the dorsal wall of the bulb abuts on the septum transversum and the liver. × 75.

inate on the anterior or ventral wall, where they are deeper and more numerous. Future widening of the spongy layer is obviously to be in this direction, and the cavity as a whole will expand mostly forward. The posterior or dorsal wall shows laterally a few shallow sinusoids, separating into individual ridges the inner muscle layer, which can be traced around the cavity as a broken line between sinusoids. The posterolateral ridge is in the position of the lower end of ridge B and may represent the lateral limb of the supraventricular crest. Further growth of the sinusoids in the right ventricle of this heart would produce a thick spongy substance and prominent trabeculae on the ventral, apical and lateral walls, and these sectors would be expanded, while ridge B would be thrust dorsally. The dorsal wall would remain relatively smooth and unexpanded. These relations are normal in the adult right ventricle.

In contrast, the similar drawing and section photograph of another embryo show a different disposition of the sinusoids (figs. 2 *b* and 3 *b*). This embryo, of 6.3 mm., is slightly smaller and presumably younger. The sinusoids are more delicate, the walls of the bulb less massive, the cavity narrower. The striking difference, however, is the fact that the sinusoids sprout from the dorsal instead of from the ventral wall of the bulb. The tip of the bulb curves to point dorsally, as is clearly shown in the photograph, and this, following the main thesis of this paper, changes the position of the sharpest bend and favors the growth of sinusoids in this unusual direction. A similar shape is shown in two of the younger models represented (fig. 1, *d* and *e*). In both of these a part of the bulb extends far dorsally toward or even under the right atrium and there makes a sharp turn. The disposition of the sinusoids in the 6.3 mm. embryo may represent the logical consequence of some of the forms shown in younger embryos.

Further growth of the sinusoids in this dorsal or reversed position must lead to internal changes in the adult right ventricle. The ventral wall will remain relatively unexpanded and smooth, while the dorsal wall will expand in all directions, the spongy substance becoming thicker and the trabeculae more prominent. Ridge B, for instance, might be expected to move laterally and ventrally and to assume the proportions of an incomplete septum projecting from the lateral wall. These conditions can be predicted by analogy from the course of normal development, but can only be hypothecated or deduced, for no such disposition was encountered in any of the older hearts examined. From present knowledge of the nature of the various portions of the heart tube, of the position of the heart in the body and of the growth forces involved in further development, deductions can be drawn, however, with a fair degree of confidence.

Expansion of the usual ventral region of deepest sinusoids in the bulb will encounter merely the thin body wall, which may be expected

to yield readily to the pressure, offering no obstacle to this growth. Expansion of the similar region of the heart of the embryo of 6.3 mm., in this case the dorsal region, will encounter the pulsating right atrium and the septum transversum or diaphragm, backed by the relatively solid liver. At this period in development the diaphragm is almost vertically disposed (Mall[17]) and must offer a formidable barrier. Meeting this, the heart itself as it expands will tend to be lifted forward. The whole heart cannot respond, however. As has been pointed out, the atriums, atrioventricular canal and left ventricle are fixed; only the bulbar portion is free for its "independent bendings." The walls of the interventricular canal may be expected, therefore, to serve as a hinge on which the forward movement of the bulb must pivot; the left wall of the bulb will be rigidly held. Thus ventral or forward movement of the lower part of the bulb can be accomplished only by a rolling motion, or rotation around a vertical axis. The lateral or right wall will be rolled ventrally or forward. Furthermore, this rotary

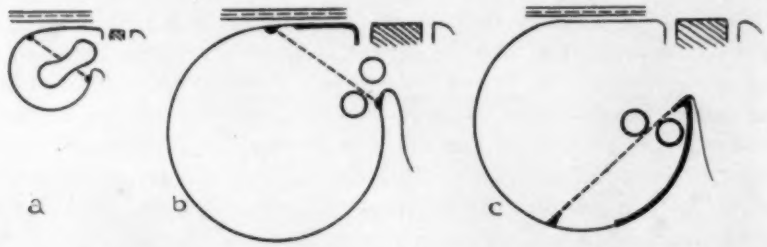

Fig. 4.—Diagrams to illustrate the effect of growth on the lower portion of the heart bulb with different dispositions of the sinusoids. Further explanation is given in the text.

motion of the lower part of the bulb will be transmitted distally or upward as a sinistral motion and will result in the detorsion of the normal dextral spiral of this portion of the heart.

These deductions can be tested by employing a tubular toy balloon, somewhat like a sausage in shape. A strip of adhesive tape applied lengthwise to one side in the collapsed state will prevent local expansion, and represent the unexpanded sector of the bulbar wall. With the normal disposition of sinusoids this would comprise the left side (interventricular canal) and the dorsal wall; with dorsally directed sinusoids, it would include the left side and the ventral wall. To represent the action in the latter case, the balloon may be held vertically with the tape toward the left, one edge resting dorsally against the side of a desk; inflation causes the anticlockwise rotation of the whole

17. Mall, F. P.: Am. J. Anat. **13**:249, 1912.

tube away from the desk as the side opposite the tape is thrust forward. The inflation naturally also lengthens the circumference of the untaped sides and spreads apart any markings made thereon. The top of the tubular balloon meanwhile is rotated sinistrally. The greater the width of the tape confining the uninflated surface, the greater is the sinistral torsion.

The mechanical principles involved are illustrated in the diagram, figure 4. Transverse sections of the embryonic bulb at the level of the bulboventricular (interventricular) canal are represented in outline, the orientation being the same as in figures 2 *b* and 3 *a*. The actual right side lies toward the left in the figure; the dorsal wall is shown at the top, pressed against the vertical diaphragm seen in section. The atriums are not included; the left ventricle is merely suggested. The two atrioventricular valves are shown as slits separated by the fused endocardial cushions. The bulbar ridges are indicated, ridge A on the interventricular septum, ridge B on the lateral wall of the bulb, and are joined by a broken line which may represent the projection of the supraventricular crest. The aortic and pulmonary valves are projected on these diagrams from a higher level. In diagram *a*, representing a stage before sinusoids appear, the vessels still show their origin from a common trunk, though suggesting its division by the fusion of the two ridges.

In diagram *b* the ventral and lateral sides of the bulb have expanded, as represented by the narrower line of the circumference. These sectors bulge ventrally (downward in the diagram) and laterally. The dorsal wall remains unexpanded, as is indicated by the broader outline. The bulbar ridges are not disturbed in their relative positions. The semilunar valves, now represented as separated, also retain their positions. They lie on either side of the crest, but are held close together by the fibers of the annuli fibrosi, a derivative of their original common bulbar wall. In diagram *b* the principal relations are roughly those of the normal adult heart.

In diagram *c* is shown the probable results of the dorsal outgrowth of sinusoids. The dorsal and lateral walls are now represented by the narrow line, the unexpanded ventral wall by the broad line. As the expanding dorsal wall meets the diaphragm, the bulb is rotated forward or ventrally. The former front wall becomes median. Ridge A is not influenced and retains its old position, but ridge B is thrust, by the lateral expansion of the dorsal wall, around the circumference to a new ventral position. The supraventricular crest joining the two ridges has changed its direction by an anticlockwise rotation of roughly a quarter circle. The aortic and pulmonary valves, retaining their previous relation to these structures, also reflect the same sinistral rotation. The aorta and the pulmonary artery will run upward side by side without torsion. This diagram, then, showing the logical consequences of the

growth of sinusoids from the dorsal wall of the bulb, represents the chief characteristics of simple transposition of the great vessels and gives an adequate explanation for the inherent detorsion.

In such a heart further changes might be expected. Other structures located normally on the dorsal or dorsolateral wall of the bulb might also be forced to separate and migrate laterally and ventrally for a certain distance, following ridge B and, like it, to become more prominent as they are undermined by deeper sinusoids; these structures include the bulboatrial ledge and the tricuspid ledge. If by their prominence and alinement with each other they form a more or less efficient septum partially shutting off an anterior portion of the right ventricle (sometimes wrongly considered as the whole of an anomalous ventricle) the pulmonary artery may receive and transmit only a small portion of the total blood flow and, following Thoma's [18] laws, may remain small. The resultant apparent stenosis would vary in intensity with the efficiency of the new septum and the general shape of the whole right ventricle. It seems unnecessary to follow Abbott [19] in considering this condition as due to fetal endocarditis. The ventral portion of the interventricular septum might be more or less deficient, partly because its right wall would be imperfectly made in the absence of the ventral bulbar sinusoids, partly perhaps because of the continued flow of blood from the left ventricle, which would have no other outlet than the interventricular canal. Absence of the ventral septum would place the pulmonary valves effectively over the left ventricle—crossed transposition. Other unrelated abnormalities of the heart might be present concurrently, and the different combinations could be classified as types. The condition of situs inversus must undoubtedly be called on for the explanation of some cases, for detorsion from the cause here postulated must be definitely limited. There seems to be no reason, however, for connecting these additional anomalies with the primary cause of detorsion.

The anomaly called "overriding of the aorta," in which the aortic valves have apparently moved slightly to the right so that the aorta drains both ventricles, is often considered as the first stage toward transposition of the great vessels. Spitzer [4] gave it as type 1. This idea seems to be incorrect. Overriding may be adequately explained as due to the commonest kind of embryologic disturbance, namely, arrest of development. This term is employed when an organ or part fails to complete the usual sequence of development and remains in a stage of immaturity. In the heart the two great vessels are separated distally

18. Thoma, R.: Untersuchungen über die Histogenese und Histomechanik des Gefäss-systems, Stuttgart, F. Enke, 1893.

19. Abbott, M. E.: Atlas of Congenital Cardiac Disease, New York, American Heart Association, 1936.

by the fusion of the ridges, as has already been said, but the separation is not completed in this way. The aorta, being a part of the bulb, arises to the right of or just above the interventricular canal, in the overriding position. The final separation of the ventricles, which really separates the aortic conus from the right ventricle, depends on the growth from the right ends of the endocardial cushions of a veil-like sheet which runs diagonally upward and forward to join both the ventral part of the interventricular septum and the aorticopulmonary septum (Sato [20]). This growth takes place in late embryonic life; its total failure, or arrest of development, leads to "overriding of the aorta"; partial failure leads to different types of septal defects. Though the membranous septum is often absent in transposition also, the two conditions are due to basically different causes.

It may well be that transposition also will prove to be ultimately due to a form of arrest of development. There may be a sequence followed by all young human hearts in which the bulb at one time shows a sharp curve pointing dorsally which later rolls forward to change its direction just before the first outgrowth of sinusoids. Delay in this final change of shape until after the appearance of sinusoids would then be sufficient to initiate the dorsal sprouting and cause the anomaly as described. In a certain sense this might be considered an arrest of development, but not as the term is usually employed. That the outgrowth of sinusoids may be delayed is shown by the fact that in one embryo of 8 mm., much older than those figured here, the sinusoids occupy as yet only a small area at the fundus of the bulb. There are so few early human embryos available for comparison, however, that no definite sequence of change of shape and appearance of sinusoids can yet be confidently established.

SUMMARY

Transposition of the aorta and the pulmonary artery is the common factor in many anomalies of the heart and as such should be the result of some slight and easily produced variation from the normal course of development. Models and drawings of human hearts of 5 mm. and less show great differences in shape, especially of the bulbar region. The lower part of the bulb is transformed into the right ventricle by the outgrowth of sinusoids, sprouting earliest and most profusely from the convex surface of some acute curve. The sharpest curve in the normal bulb points downward and forward, and the spongy substance of the right ventricle, therefore, is chiefly on the ventral and apical walls. In a few of the younger hearts shown the sharpest curve points downward and backward or dorsally, and in one human embryo studied the sinusoids, which are just developing, are found on the apical and dorsal walls of the ventricle. Continued growth in this dorsal position

20. Sato, S.: Anat. Hefte **50**:193, 1914.

would meet the opposition of the diaphragm, and the right ventricle would be forced ventrally. Since the left wall of the embryonic right ventricle is attached to the interventricular canal, ventral displacement can be accomplished only by a rotary anticlockwise motion, which when transmitted to the distal bulb would counteract the normal dextral torsion and result in transposition.

The expansion of the dorsal and lateral walls at the expense of the ventral wall might also result in the displacement ventrally of the supraventricular crest, the bulboatrial ledge and the anterior tricuspid ledge and in their intensification to form more or less complete septums across the ventricle. The ventral pouch bordered by such septums is not, however, the true right ventricle. Stenosis of the pulmonary artery may depend on the efficiency of such septums. Failure of the ventral sinusoids may cause the malformation or absence of the ventral interventricular septum, resulting in "crossed transposition."

"Overriding of the aorta" is due to arrest of development, as all mammalian embryonic hearts pass through this stage before the final separation of the ventricles by the growth of the membranous portion of the interventricular septum. This condition has no connection with transposition.

The main deductions of this paper rest chiefly on the tenuous basis of conditions found in a single human embryo and are therefore submitted as a theory rather than as a proved exposition of the cause of the anomaly. Yet they trace the logical mechanical effects of growth forces on an observed variation from normal and show how these would result in transposition and its many accompanying changes in the heart.

THE ADRENAL CORTEX IN ESSENTIAL HYPERTENSION

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In 1941 Rinehart, Williams and Cappeler [1] reported that adenomatous hyperplasia of the adrenal cortex was almost regularly found in association with essential hypertension. The adrenal condition was described by them as a gross irregular thickening and nodularity of the cortex with microscopic irregularity of the cortical cords, which were hyperplastic and generally well filled with fine lipoid droplets. They also found that the mean weight of the adrenals was greater by 4.2 Gm. in a group of cases of essential hypertension than in a control series.

In view of the importance of these observations in relation to the problem of essential hypertension, it seemed worth while to attempt to confirm them in an independent study.

MATERIALS AND METHODS

In a series of unselected routine autopsies on adult subjects the adrenal glands were removed intact, roughly dissected free of surrounding fat and then fixed in a dilute solution of formaldehyde U. S. P. (1:10). After fixation they were carefully freed from adherent fat and the two adrenals from each subject weighed together. Paraffin sections were made from a block cut transversely though the thickest part of each adrenal and stained with hematoxylin and eosin. The routine histologic sections of the kidneys, the heart and other organs were available for study, as well as records of the weights of these organs.

Adrenal glands from persons with glomerulonephritis or pyelonephritis and adrenal glands which showed evidence of tuberculosis, cancer metastasis, hemorrhage or extensive edema were not included in this series.

In the work of Rinehart and associates [1] the criteria for the selection of cases of essential hypertension were clearly defined as regards the weight of the heart but the limits of blood pressure were not clearly stated. In the present series, the cases classified as those of essential hypertension were selected with a view to excluding all doubtful instances, and to this end the rigid criteria of Moritz and Oldt [2] were applied, namely:

Hypertensive patients

Males { Heart weight 450 Gm. or more.
Blood pressure 160 systolic and 90 diastolic or 150 systolic and 100 diastolic or higher.

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1. Rinehart, J. F.; Williams, O. O., and Cappeler, W. S.: Arch. Path. **32**:169, 1941.

2. Moritz, A., and Oldt, M. R.: Am. J. Path. **13**:679, 1937.

Females { Heart weight 350 Gm. or more.
Blood pressure 160 systolic and 90 diastolic or 150 systolic and 100 diastolic or higher.

Nonhypertensive patients

Males { Heart weight less than 400 Gm.
Blood pressure not over 140 systolic and 90 diastolic.

Females { Heart weight less than 300 Gm.
Blood pressure not over 140 systolic and 90 diastolic.

If severe coronary arteriosclerosis was present, a heart weight in excess of 500 Gm. was required for acceptance of the case as one of essential hypertension.

Many cases fell into an intermediate group because the heart weight or the blood pressure was such that the condition could not be classified either as hypertensive or as nonhypertensive.

Cases of cardiac hypertrophy associated with inflammatory heart disease or pulmonary disease were grouped separately.

Combined Weights of Adrenal Glands

Group	Cases	Mean Weight of Adrenals	Standard Deviation	Standard Error of Mean
Nonhypertensive conditions (controls)	50	13.08	3.3	.47
Intermediate conditions	21	13.11	2.7	.59
Essential hypertension	19	14.02	3.4	.78
Cardiac hypertrophy with inflammatory heart disease or pulmonary disease	12	13.58	3.8	1.1

OBSERVATIONS

In the accompanying table the average weights of the adrenal glands in the four groups of cases are tabulated. Although among the cases studied the average weight is very slightly greater in the cases of essential hypertension than in the other groups, this small difference is not statistically significant.

In none of the groups of cases shown in the table was the gross appearance of the adrenal cortex sufficiently distinctive to permit differentiation between groups. Cortical adenoma was present in 4 nonhypertensive cases, in 2 cases of the intermediate group, in 3 cases of essential hypertension and in none of the cases of cardiac hypertrophy associated with inflammatory heart disease or pulmonary disease. The largest tumor diagnosed as cortical adenoma measured 2.5 cm. in diameter and the smallest 0.6 cm. in diameter. In 2 cases cortical adenoma was bilateral and in 1 case a single adrenal presented two tumors of this classification.

The adenomatous hyperplasia of the adrenal cortex described by Rinehart and co-workers [1] as a characteristic and an almost constant finding

in cases of essential hypertension was not encountered constantly in the hypertensive cases in this study and was frequently found in the cases of other groups. The microscopic appearance of generalized irregular tortuosity of the cortical cords composed of cortical cells well filled with fine lipoid droplets was present in 4 of the nonhypertensive controls, in 6 of the intermediate group, in 5 of the cases of essential hypertension and in 2 of the cases of cardiac hypertrophy associated with inflammatory heart disease or pulmonary disease. This microscopic picture was universally present in areas of cortical adenoma. In 3 of the cases presenting cortical adenoma, the remaining adrenal cortical tissue presented a similar appearance. Although 5 of the cases of essential hypertension presented the microscopic appearance described by Rinehart and co-workers [1] as characteristic of adenomatous hyperplasia of the adrenal cortex, it is important to note that the remaining 14 cases of essential hypertension presented the normal cortical structure. Only 4 cases of the nonhypertensive group presented the microscopic finding of adenomatous hyperplasia. However, it is significant that the appearance of the adrenal cortex in these cases differed in no essential respect from that found in the cases of the other groups.

COMMENT

It is evident from the foregoing observations that the findings of Rinehart and associates [1] were not confirmed in this study. Although the number of cases of essential hypertension in this series is slightly smaller than that in the series studied by those authors it must be emphasized that they were selected with the greatest care, and strict qualifications were required for inclusion in the group. It is not at all clear that all of the 26 cases classified as instances of essential hypertension in the series collected by Rinehart and co-workers would bear the application of the same rigid criteria.

SUMMARY

In a series of unselected routine autopsies on adult subjects the adrenal glands were removed, fixed in dilute solution of formaldehyde, carefully dissected free of surrounding fat and the adrenals from each subject weighed together. Paraffin sections were made from a block cut transversely through the thickest part of each adrenal and stained with hematoxylin and eosin.

Cases of essential hypertension and a group of nonhypertensive controls were segregated on the basis of rigid criteria. Other cases were appropriately grouped in separate categories.

The average weight of the adrenal glands in cases of essential hypertension is not significantly higher than that in nonhypertensive control cases.

Nodular or adenomatous hyperplasia of the adrenal cortex is not regularly found in association with essential hypertension, and it occurs with considerable frequency in nonhypertensive cases.

The microscopic appearance of tortuosity of the adrenal cortical cords and abundant deposition of fine lipoid droplets in the cortical cells is not consistently associated with the gross finding of irregularity and nodularity of the adrenal cortex.

The observations of Rinehart and associates [1] are not supported by the findings in the present study.

PRODUCTION OF CIRRHOSIS OF THE LIVER IN RATS BY FEEDING LOW PROTEIN, HIGH FAT DIETS

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During the past two years experiments have been reported on the production of cirrhosis of the liver by dietary methods. György and Goldblatt [1] observed various pathologic changes in the livers of rats fed a diet deficient in parts of the vitamin B complex. In 4 animals of their large experimental group "perilobular and condensation fibrosis" developed, and in 2 "this change was so severe as to warrant the diagnosis of localized cirrhosis." One of us (Blumberg [2]) reported that feeding of wheat germ oil prepared by ether extraction produced diffuse nodular cirrhosis of the liver in 7 rats. These rats received large amounts of the oil (3 to 5 cc. per day) for two hundred and forty-three days or longer. The diet was notably high in fat and low in protein. Further investigations have been conducted with wheat germ oil and also with commercial corn oil in order that the development of the cirrhotic changes might be studied. A description of the pathologic process is presented in this report.

While these investigations were in progress, additional publications appeared on the dietary production of hepatic cirrhosis in various species. Rich and Hamilton [3] produced cirrhosis of the liver in rabbits on deficient diets and reported prevention of the changes by yeast. By means of diets high in fat Chaikoff and Connor [4] produced cirrhosis in 3 dogs. Spellberg and Keeton [5] observed cirrhosis apparently of

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1. György, P., and Goldblatt, H.: J. Exper. Med. **70**:185, 1939.
2. Blumberg, H.: Pub. Health Rep. **55**:531, 1940.
3. Rich, A. R., and Hamilton, J. D.: Bull. Johns Hopkins Hosp. **66**:185, 1940; Tr. A. Am. Physicians **55**:133, 1940.
4. Chaikoff, I. L., and Connor, C. L.: Proc. Soc. Exper. Biol. & Med. **43**: 638, 1940.
5. Spellberg, M. A., and Keeton, R. W.: Am. J. M. Sc. **200**:638, 1940.

dietary origin in 1 guinea pig and in 1 rabbit. Earle and Victor [6] obtained hepatic cirrhosis in 7 rats of a group fed cystine in the excessive concentrations of 5 and 10 per cent of the diet. György and Goldblatt [7] reported injury of the liver (necrosis, cirrhosis) in rats fed a diet containing 10 per cent casein and 22 per cent fat, with thiamine, riboflavin, pyridoxine and pantothenic acid supplied as members of the vitamin B complex. In experiments related to the investigations to be described in this article, Blumberg and McCollum [8] produced hepatic cirrhosis in rats on diets high in lard and low in protein (casein) and found that the cirrhotic changes were prevented by the addition of choline. Practically complete prevention was also obtained by incorporating 10 per cent of yeast in the diet or by increasing the casein level to 24 per cent, while some beneficial effect was noted in preliminary experiments with methionine, an essential amino acid in which casein is relatively rich. Lillie, Daft and Sebrell [9] have reported cirrhosis in rats on a diet low in both protein and fat and perhaps in other factors, as well as in rats on the deficient diet plus alcohol.

EXPERIMENTAL PROCEDURE

Albino rats of the Buffalo, P. H. and Wistar stocks were used for these investigations. The animals were allowed to reach weights of 125 to 200 Gm. before being started on the experimental diets. The basal diet used was the same stock mixture that was required for the previous experiments on wheat germ oil feeding [2] and had the following composition: cracked corn, 60 parts; rolled oats, 15; meat scrap, 14; skimmed milk powder, 10; sodium chloride, 1. Cod liver oil was added to the mixture at a level of 1.5 per cent. A supplement of carrots (without tops) was given once a week, each rat receiving about one third of a medium-sized carrot. This basal diet had a protein content of about 18 per cent and was adequate for approximately normal growth. The wheat germ oil was prepared by twenty-four hour ether extraction of wheat germ, as previously described.[2] The corn oil was a commercial product (Eimer & Amend) prepared by expression.

Wheat Germ Oil Feeding.—The wheat germ oil was fed as a supplement to the basal diet, at a level of 3 to 5 cc. per rat per day. The amount fed, which varied with the weight of the rat, was adjusted so as to maintain the animal at an approximately constant weight. Under these conditions the diet contained about 50 per cent fat and 10 per cent protein. At various intervals of time, as the animals died or were put to death, the livers were removed and fixed in 4 per cent solution of formaldehyde or in Zenker's fluid.

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6. Earle, D. P., Jr., and Victor, J.: J. Exper. Med. **73**:161, 1941.

7. György, P., and Goldblatt, H.: Proc. Soc. Exper. Biol. & Med. **46**:492, 1941.

8. Blumberg, H., and McCollum, E. V.: Science **93**:598, 1941; to be published.

9. Lillie, R. D.; Daft, F. S., and Sebrell, W. H., Jr.: Pub. Health Rep. **56**:1255, 1941.

The results are shown in the upper part of the accompanying table. Cirrhosis was produced in 15 animals, the earliest case being observed after one hundred and thirty-nine days of feeding the oil supplement. The livers of animals examined post mortem previous to this time presented varying degrees of fatty change. By an occasional temporary decrease in oil dosage a few of the animals were kept alive for more than four hundred days to provide material for a study of the effects of prolonged cirrhosis. Diffuse nodular cirrhosis was produced in all three strains of rats. Ascites with hydrothorax was observed in 2 animals.

Corn Oil Feeding.—As a means of determining whether or not the production of cirrhosis in the previous experiments was due to a factor peculiar to wheat germ oil or to the type of ether-extracted oil used, experiments were conducted with a commercial expressed corn oil. A group of 7 Buffalo and P. H. rats was fed a diet consisting of the basal ration plus 36 per cent corn oil, a diet in which the fat was approximately 41 per cent and the protein 12 per cent. The 6 animals which were on the diet for two hundred and fifty-eight to three hundred and seventy-two days had only fatty changes in the liver, without cirrhosis, as shown

Cirrhosis of the Liver in Rats on Wheat Germ Oil and Corn Oil Diets

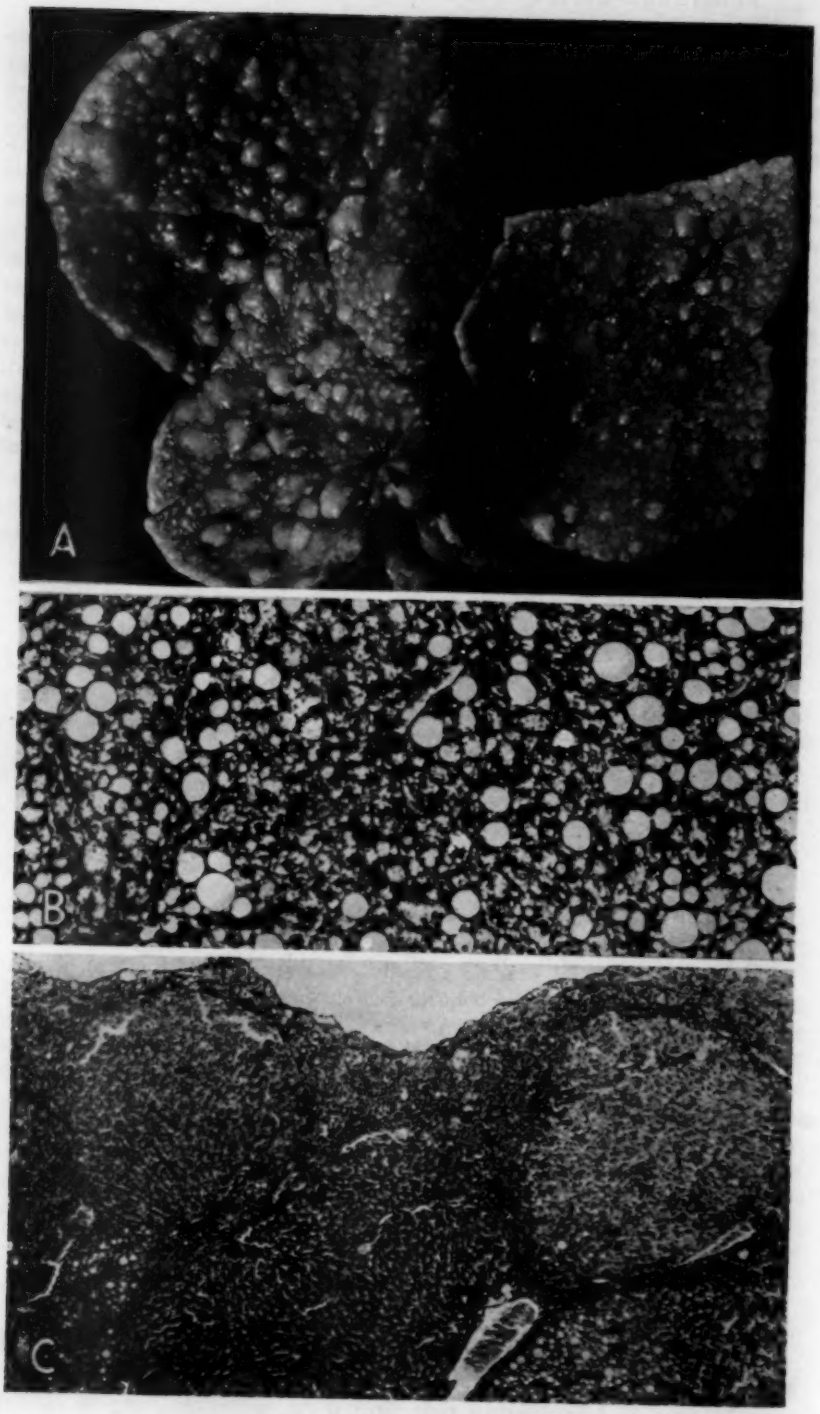
Oil Supplement to Diet	Days on Diet	Rats	Rats with Cirrhosis
Wheat germ oil (3 to 5 cc. per day)	0-100	9	0
	100-150	5	2
	150-200	4	2
	200-250	5	3
	250-300	4	4
	300-450	4	4
Corn oil (36 per cent)	250-300	1	0
	300-400	5	0
	400-450	1	1
Corn oil (3 to 4 cc. per day)	150-200	2	0
	200-250	2	1
	250-300	4	3
	300-350	2	2

in the middle section of the table, whereas the 1 animal that was maintained for four hundred and thirty days showed early cirrhosis of the liver.

In order to repeat the conditions of the experiment in which wheat germ oil was used, a group of 10 rats of both the Buffalo and the P. H. strain was fed the basal ration with corn oil supplements at the level of 3 to 4 cc. per rat per day. This diet, like that with the wheat germ oil ration, contained approximately 50 per cent fat and 10 per cent protein. The results of this experiment are shown in the lower part of the table. Diffuse nodular cirrhosis was first observed after two hundred and seventeen days of feeding the oil supplement, while animals killed earlier showed extensive fatty change. Cirrhosis was produced in 6 animals of this corn oil group, in rats of both Buffalo and P. H. strains. Ascites with hydrothorax occurred in 3 animals. This experiment demonstrated that the production of cirrhosis was not peculiar to wheat germ oil feeding.

PATHOLOGY

On gross inspection during the early stages of feeding of either wheat germ oil or corn oil, the liver was of approximately normal size, smooth, firm and frequently pale brown or sometimes yellow. With



(Explanation of plate on opposite page)

the development of cirrhosis the surface became finely granular and the cut surface distinctly more firm. These changes were frequently noted first in the small caudate lobe and were usually more pronounced here even when the cirrhotic process involved all the other lobes. After two hundred and forty-three days or more of oil feeding a few larger nodules, ranging from 2 to 5 mm. in diameter might be seen projecting above the surface (fig. *A*). They varied in color from the yellow-brown of the surrounding tissue to yellow-white and grayish white, and occasionally the surface appeared unusually vascular. Jaundice was not observed, and there were no constant or pertinent lesions of organs other than the liver.

Histologically, the lesions may conveniently be divided into three categories as follows: (1) the fatty stage, (2) the stage of fatty change with minimal cirrhosis and (3) the stage of diffuse nodular cirrhosis.

In the fatty stage (fig. *B*), which was observed as early as fifty-four days after the beginning of the experiment, practically every hepatic cell was occupied by a single large or several smaller fat droplets, which stained red with sudan IV. Milder degrees of this process were observed, but there was no constant spatial relationship of the fat to the hepatic lobule. Within the same section the fat might be concentrated in the central zones in one area and in the portal zones in another area. No significant changes other than the presence of fat were noted. The parenchymal cells uninvolved in the fatty changes appeared essentially normal.

In the second stage the fatty process remained qualitatively unchanged although varying somewhat in degree. The characteristic feature of this stage was a proliferation of fibroblasts and mononuclear cells chiefly in the portal areas and perilobular tissue, with occasional minor extensions into the lobule. The fibroblasts were thin and elongated and possessed relatively little cytoplasm; very few collagenous fibers were laid down. With the exception of the invasion of the lobules by the thin fibroblasts, the normal structural pattern of the liver remained intact. No significant changes were observed in the bile ducts.

The final stage (fig. *C*) represented a diffuse and usually still active proliferation of fibroblasts which had completely disrupted the hepatic

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EXPLANATION OF PLATE

A, diffuse cirrhosis showing typical hobnail appearance; × 2.

B, severe fatty change in a liver after fifty-four days of wheat germ oil feeding. Hematoxylin-eosin; × 120.

C, cirrhotic liver showing large nodules of hepatic cells replacing the normal lobular pattern. Note the variation in the distribution of fat. Hematoxylin-eosin; × 65.

pattern and enclosed in its meshes hepatic nodules of varying sizes and configurations. Frequently small nests or even single parenchymal cells were caught in the proliferating connective tissue. The connective tissue itself was still highly cellular and generally the collagenous fibers were relatively thin. There was remarkable variation in the fat content of the hepatic nodules, some of these being completely devoid of visible fat while others showed practically every cell vacuolated. The large nodules noted on gross inspection were composed of cords of hepatic cells, often with smooth acidophilic cytoplasm, widely separated by sinusoids; the nodules were bounded by a thin rim of connective tissue. It was not possible to decide on histologic grounds whether these structures represented merely regenerative hyperplasia or were true hepatoma. Proliferation of bile ducts was commonly found, and in a few instances large focal areas were occupied by masses of proliferating and poorly formed ducts. The lumens of the ducts were usually empty, and no indication of biliary stasis was observed. An interesting and striking feature of several livers in this stage was the occurrence in hematoxylin-eosin preparations of small masses of brilliant canary yellow globular pigment,[10] which was for the most part extracellular although occasionally it appeared within macrophages. The masses of pigment were found within the proliferating fibrous tissue and sometimes in close relation to blood vessels. The material stained brilliant red with 2 per cent aqueous basic fuchsin, but failed to react to Turnbull's blue. In sections stained by Verhoeff's method for tubercle bacilli, it retained the fuchsin stain. In view of these properties, particularly the reaction to 2 per cent aqueous basic fuchsin, the pigment may be regarded as hemofuscin.

COMMENT

By addition of the large oil supplements to the basal ration, the protein content was reduced to about 10 per cent and the fat increased to about 50 per cent. Also, the caloric value of the diet was raised because of the increased proportion of fat. In view of the composition of the diet and the fatty changes in the liver preceding the appearance of the cirrhotic changes, it seems likely that the low level of protein in the diet, aided by the high fat content, was of importance in the production of the cirrhosis. The fat content of the liver is known to increase under these conditions in the absence of sufficient choline, shown by Best,

10. A similar pigment has been observed in larger quantities accompanying cirrhotic changes in the liver in subsequent work by Blumberg and McCollum.[8] A somewhat similar type of pigment has also been seen by J. E. Edwards and J. White (J. Nat. Cancer Inst. **2**:157, 1941) in cirrhosis of the liver produced by feeding rats p-dimethylaminoazobenzene (butter yellow).

Hershey and Huntsman [11] to be a lipotropic factor, and with diets low in the essential amino acid methionine, as demonstrated by Tucker and Eckstein.[12] According to Connor,[13] the process of prolonged fatty infiltration of the liver may lead to cirrhosis, as is seen in diabetes and in chronic alcoholism in man.

It is interesting that some of the cirrhotic livers exhibited areas of regeneration bearing a morphologic resemblance to hepatoma.

SUMMARY

Cirrhosis of the liver, usually of the diffuse nodular type, was produced in three strains of rats fed large quantities (3 to 5 cc. per rat per day) of wheat germ oil or of corn oil for approximately two hundred to four hundred days. The sequence of changes appears to be that of prolonged fatty infiltration followed by cirrhosis. The production of the cirrhosis seems to depend, at least in part, on a deficiency of protein in the diet, aided by a high fat content.

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11. Best, C. H.; Hershey, J. M., and Huntsman, M. E.: Am. J. Physiol. **101**:7, 1932.
12. Tucker, H. F., and Eckstein, H. C.: J. Biol. Chem. **121**:479, 1937.
13. Connor, C. L.: Am. J. Path. **14**:347, 1938.

THE PARATHYROID GLAND IN INFANCY

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Many investigators in recent years have studied the parathyroid glands from the standpoints of physiology and pathology. Adequate bibliographies and historical reviews are to be found in the literature, particularly in the monograph by Shelling [1] and the papers of Castleman and Mallory,[2] Gilmour,[3] Morgan [4] and Norris.[5] These authors and others have described parathyroid glands in health and in disease but have in no instance systematically compared the parathyroid glands in a large series of infants and children. Such a study is the basis for this report.

Sections from the parathyroid glands of 235 infants and children were obtained from autopsy material of the Children's Hospital. About 200 of the specimens were found in the routine postmortem material, and about 35, by planned dissection at autopsies. The former group consisted for the most part of single parathyroid glands included accidentally in sections of the neck structures, fixed in Zenker's fluid. In the latter group, more than one gland was almost invariably found, gross inspection and measurement were possible, and special fixatives and stains could be employed as supplements to the routine procedures. The present study is based principally on the histologic examination of single sections of single glands in routine tissue, but in no case in which additional material was available were the histologic variations sufficient to cast doubt on the adequacy of the sampling.

The method of study was essentially a systematic comparison of sections arranged in order of age. Criteria for histologically normal glands were established, and a small group of 35 glands were put aside as sufficiently varied from the remainder to warrant further study. Of the 200 "normal" glands, a few exhibited incidental aberrations obviously due either to local conditions, such as suppuration, or to systemic conditions, such as amyloidosis or hemosiderosis.

THE NORMAL PARATHYROID GLAND

The histologic appearance of the normal parathyroid gland has been well described. Nevertheless some confusion exists, particularly con-

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1. Shelling, D.: The Parathyroids in Health and Disease, St. Louis, C. V. Mosby Company, 1935.

2. Castleman, B., and Mallory, T. B.: Am. J. Path. **13**:4 and 553, 1937.

3. Gilmour, J. L.: J. Path. & Bact. **44**:431, 1937; **45**:507, 1937; **46**:133, 1938; **48**:187, 1939.

4. Morgan, J. R. E.: Arch. Path. **21**:10, 1936.

5. Norris, E. H.: Contrib. Embryol. (no. 159) **26**:249, 1937.

cerning nomenclature, and so a brief statement as to the terms to be used and as to the criteria established for the age period studied is warranted. Despite a multiplicity of terms, there is general agreement that the glandular epithelium consists of several cell forms, probably representing different metabolic phases of a single epithelial cell type. The predominant cells are called synonymously principal, chief or parietal cells. Depending on the cytoplasmic reaction to ordinary stains, they are further classified as pale, dark or clear (virtually unstained). Those clear cells that are increased in size are called water clear, swollen or large vesicular cells. Large cells with prominent oxyphil cytoplasmic granules are called pale oxyphil cells. Small cells with dark eosin-stained cytoplasma are called dark oxyphil cells.

The terms "chief cell" and "water clear cell" will be used in this report. The chief cells are of regular rather than eccentric shape and usually of uniform shape (round, oval, cuboidal, columnar or polygonal) in the same cell cord. They vary in size from about 6 to 12 microns, and the nucleus is one third or more of the cell size and either central or polar in position. Dark chief cells have many fine granules, pale chief cells have a few peripheral granules, and clear chief cells have virtually no granules. Water clear cells are of irregular, eccentric shape, are more than 12 microns in diameter, with eccentrically placed nuclei one third or less of cell size, and usually sharply defined cell margins. Frequently the plane of section fails to include the nucleus, because of the large cell size.

In the normal gland (fig. 1 *A*) the epithelium is arranged in syncytial cords separated by varying amounts of connective tissue into ill defined lobes and lobules. The chief cells occasionally form pseudoalveoli, line irregular cystlike spaces or may be alined in a row bordering a blood vessel or connective tissue bundle. In some glands there is marked capillary hyperemia; in others, marked dilatation of perivascular spaces. Hemopoietic activity is present in only a few glands of early infancy. Occasional hemosiderin deposits are to be found in the very young glands, but no severe recent or old hemorrhage. Several trends are to be noted as the parathyroids of older children are compared with those of the very young. The cytoplasm tends to become darker in staining, the stroma in the interlobar septums becomes more prominent, and adipose tissue makes its appearance in the connective tissue.

PARATHYROID HYPERTROPHY

Significant alterations from the normal histologic appearance were found in the glands of 35 infants and children. These changes involved all the parathyroid tissue of a given patient in the same manner. As will be shown, enough similarities were observed between the glands of the group to warrant the designation of their condition as parathyroid hypertrophy.

In a large number the only discernible change from the normal was the replacement of the usual chief cell epithelium by large water clear cells (fig. 2 *B*). In several glands (fig. *a*), the epithelium was water

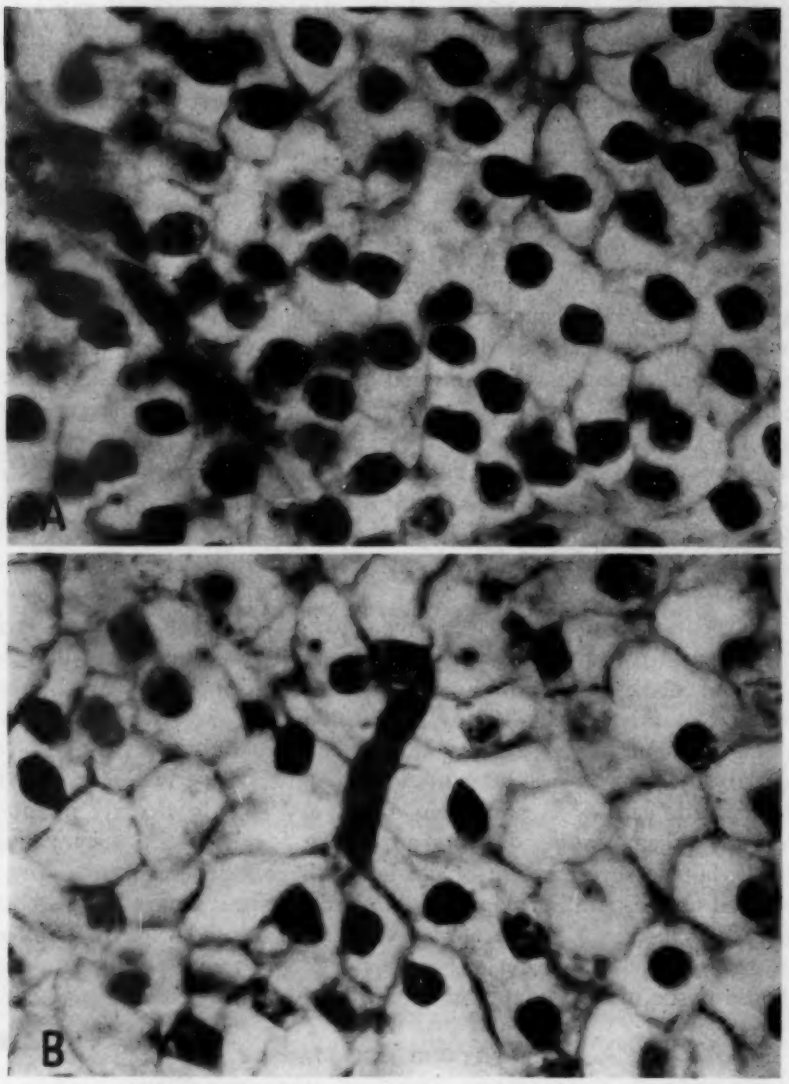

Fig. 1.—*A*, normal parathyroid gland of a boy aged 6 hours, showing chief cells arranged in syncytial cords and pseudoalveoli; hematoxylin and eosin; × 450. *B*, hypertrophy of parathyroid gland in a boy aged 9 days, characterized by water clear cells arranged in syncytial cords; hematoxylin and eosin; × 450.

clear in type but in addition, the cell cords were tortuous and interrupted frequently by intralobular stroma or capillaries. In other glands,

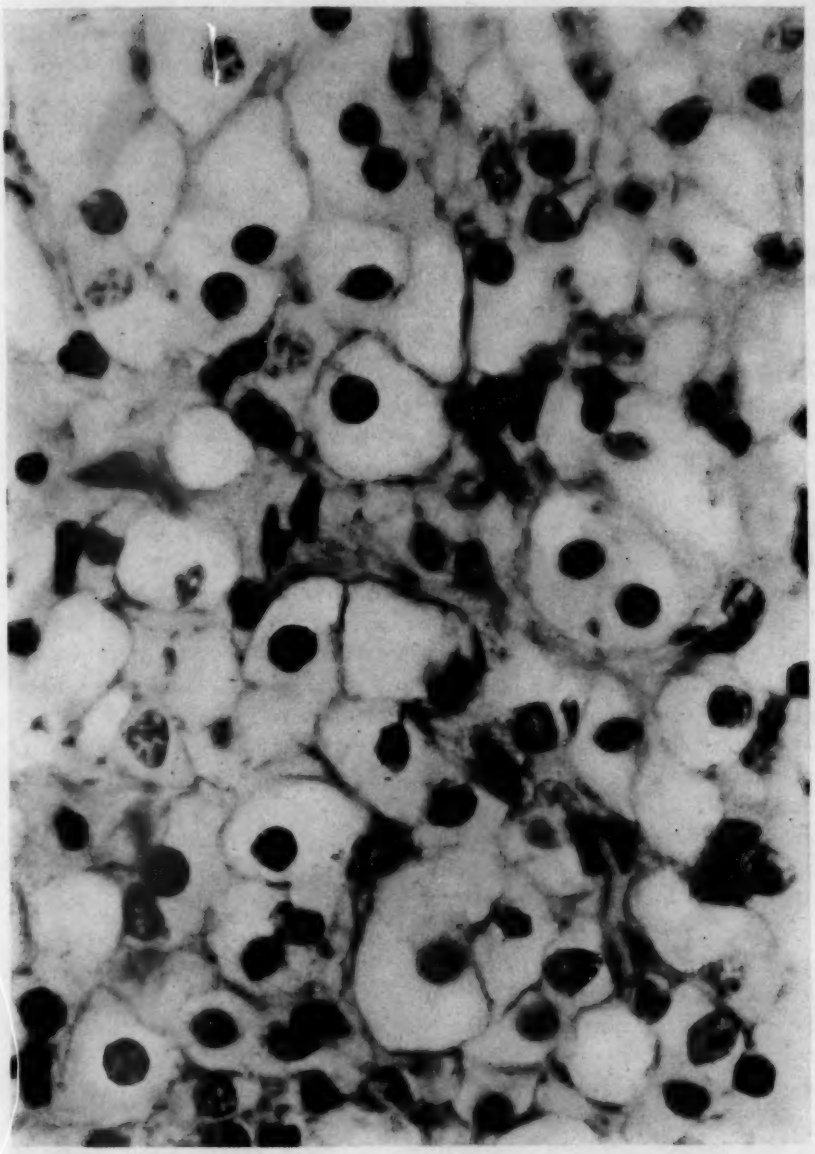

Fig. 2.—Hypertrophy of parathyroid gland in a boy aged 4 months with rickets, showing water clear cells arranged in tortuous and interrupted cords; hematoxylin and eosin; × 425.

cell cords of the type just described were noted, but the epithelium was strikingly varied in character—cells of irregular size and shape were present in most cell cords. In another group of glands (fig. 3) the normal structural pattern was replaced by dense cell sheets in the periphery, and by dense cell islands and clusters in the central two thirds, separated by delicate encircling stromal partitions and containing cells of normal size but varying shape and stain. Common to all the glands were a lack of prominence of large interlobar septums and a paucity of adipose tissue (significant in older patients). Many sections were considerably larger in diameter than the largest normal glands available for comparison at the same age. Other sections, although not measurably larger, were of the histologic type associated with measured hypertrophy in the adult literature.

Analysis of the autopsy protocols of the 35 patients reveals that 7 children had severe renal disease, 8 children rickets and 20 children no abnormality of either the skeletal or the urinary system. In each instance these facts were checked by microscopic sections of the long bones and kidneys.

Parathyroid glands were studied from 25 children with major renal lesions at autopsy. In 18 cases the glands were essentially normal. The renal lesions were either of recent origin or of a nature insufficient to have caused protracted renal failure. Included in this group were 4 cases of long-standing nephrosis. The 7 hypertrophic parathyroid glands were uniformly associated with a history of severe or protracted renal insufficiency. In 1 case generalized skeletal changes consistent with the diagnosis of renal rickets were present. In the other 6 cases, no abnormalities were noted on gross examination of the skeleton or on microscopic section of the ribs. The histologic picture in 4 cases, including 1 case of renal rickets, was that of dense cell clusters with encircling partitions. In 3 cases there was marked tortuosity of the cords, in 2 of which the epithelium was varied in type, while in the other it was uniformly water clear.

Parathyroid glands were studied from 9 children with rickets at autopsy. In only 1 case, that of early rickets in a child with osteomyelitis, was the gland normal. In a case of healing rickets, portions of the gland were normal, whereas in other areas the condition histologically resembled hypertrophy. In 3 cases there was predominance of water clear cells in tortuous epithelial cords. In 4 cases, including 1 case of treatment-resistant rickets, the epithelium was strikingly varied in type. The histologic observations on three glands from the child with treatment-resistant rickets included a change not noted in any other glands in the series, namely, prominence of collagen bundles dispersed throughout the fine intralobular septums. Dr. B. Castleman and Dr. F. Albright of the Massachusetts General Hospital supplied a section of parathyroid gland obtained for biopsy from a child with

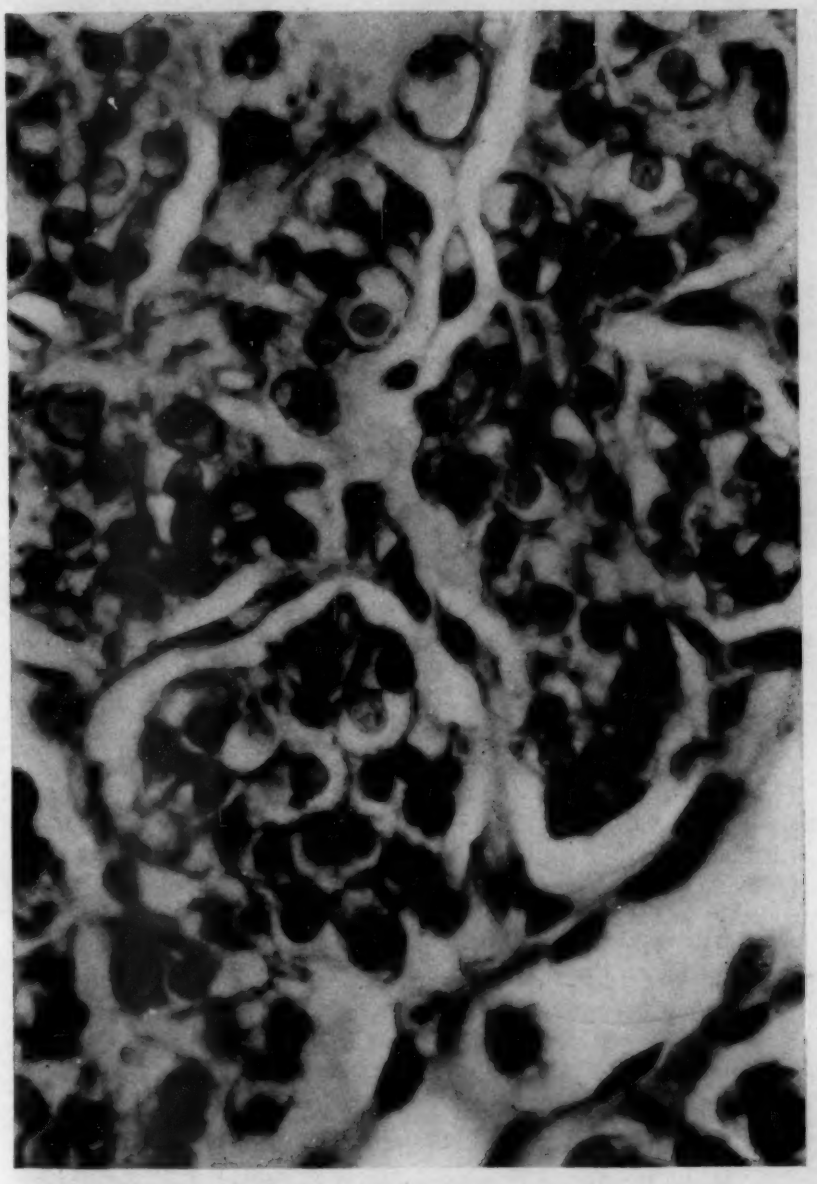

Fig. 3.—Hypertrophy of parathyroid gland in a girl aged 3 years with renal rickets, showing cells of varied size and shape arranged in dense masses and clusters; hematoxylin and eosin; × 425.

treatment-resistant rickets.[6] In this section a variety of cell size and shape within tortuous epithelial cords was noted, with no increase in the collagenous stroma.

Parathyroid hypertrophy in human rickets has been described by Erdheim,[7] Ritter[8] and Pappenheimer and Minor.[9] These authors agreed that the glands are grossly enlarged but disagreed as to the histologic changes present. As quoted by Pappenheimer and Minor,[9] Erdheim concluded that he could not "tell from the microscopic appearance of a child's parathyroid whether it came from a rachitic or a nonrachitic child," although he did note an increase in the size and the number of cells in the glands of the rachitic rat. Ritter reported 12 cases, of which 10 fall into the age group of this series, and was impressed by the hyperemia and in some cases by the marked increase in the connective tissue surrounding individual cell cords. In 6 of 10 cases the predominant cell was normal in size with dark cytoplasm. In 4 cases "clear swollen cells" were predominant or present in abnormal number. In 1 case of very severe rickets in a 2 year old girl the condition described closely resembled the type with dense cell islands herein described. Pappenheimer and Minor reported on rickets in children between 2 months and 16 months of age. Although enlargement in the size of the glands was noted, they found no difference between the glands of these children and those of nonrachitic children with respect to cell type, hyperemia or connective tissue. In the light of the material presented here it appears likely that the apparent contradiction in the various observations recorded is the result of differences in the degree of hypertrophy of the material and of inadequate comparison with normal and with other hypertrophied glands.

In 20 cases parathyroid hypertrophy was not associated with either rickets or renal disease. In every gland the epithelium was predominantly water clear in type, and in all but a few the cell cords were not otherwise abnormal. In these few a moderate increase in tortuosity was observed. Careful study of the autopsy protocols failed to reveal any common pathologic process. However, the age distribution of the children in this group was extremely interesting. Fourteen were less than 1 month of age; 11 were between 3 days and 10 days of age. Of 53 parathyroid glands studied from infants between the ages of 1 hour and 30 days, none of 20 from those between the ages of 1 hour and 72 hours were hypertrophied, but 11 of 13 from infants between the ages of 72 hours and 10 days were hypertrophied, and

6. Albright, F.; Butler, A., and Bloomberg, E.: Am. J. Dis. Child. **54**:529, 1937.

7. Erdheim, J.: Denkschr. d. Akad. d. Wissensch. **90**:363, 1914.

8. Ritter, C.: Frankfurt. Ztschr. f. Path. **24**:137, 1920.

9. Pappenheimer, A. M., and Minor, J.: J. M. Research **42**:391, 1921.

3 of 20 from those between the ages of 10 days and 30 days. Five more of the hypertrophic glands were from infants between 1 and 6 months of age, and 1 was from a patient 12 months of age.

The concentration of parathyroid hypertrophy in the neonatal period is interesting in the light of the observed transient hypocalcemia and hyperphosphatemia in the normal newborn infant, and in relation to the concept of physiologic hypoparathyroidism as the mechanism for neonatal tetany.[10] Study of considerably more than the present meager material is necessary before one ought seriously to speak of physiologic parathyroid hypertrophy of the newborn.

A review of the histologic observations on the 35 hypertrophic parathyroid glands suggests the genesis of parathyroid hypertrophy. The initial morphologic response to the stimulus, be it associated with a possible physiologic disturbance of the newborn or the pathologic state of renal failure or active rickets, is marked swelling of the epithelium. Subsequently, the epithelial cords become tortuous, and there is an increase in the fine intralobular stroma. Hyperplasia is very likely, since there is a definite increase in the diameter of the glands in many instances. Continued stimulation is then followed by a tendency for the swollen epithelial cells to shrink. Finally, the cords are again composed of small cells but now arranged densely into clusters separated by well defined stroma partitions.

SUMMARY

A study of the parathyroid glands from 235 unselected infants and children revealed 35 abnormal glands, each exhibiting diffuse epithelial hypertrophy or hyperplasia.

Hypertrophy of the parathyroid glands was present in 8 of 9 patients with rickets. Hypertrophy of the parathyroid glands was present in 7 of 25 patients with severe renal disease—in every patient in whom impairment of renal function had been severe and prolonged. In 20 patients the hypertrophy was not associated with rickets or renal disease; 11 of these patients were 3 to 10 days of age. A study of 53 patients between birth and 30 days of age revealed 14 cases of hypertrophy of the parathyroid glands—in no case associated with renal disease or rickets.

The age distribution of these hypertrophic glands suggests that hypertrophy of the parathyroid glands may be physiologic in the newborn period.

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10. Bakwin, H.: J. Pediat. **14**:1, 1939. Denzer, B. S.; Reiner, M., and Weiner, S. B.: Am. J. Dis. Child. **57**:809, 1939. Barnes, D. J., and Munks, B.: Proc. Soc. Exper. Biol. & Med. **44**:327, 1940.

EQUINE ENCEPHALOMYELITIS (WESTERN) IN MAN—A HISTOLOGIC AND ANATOMIC STUDY

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For many years in the agricultural regions of the Middle and Far West there have occurred outbreaks of a serious disease among farm horses, variously designated as Kansas-Nebraska horse plague, forage poisoning, botulism and cerebrospinal meningitis. Following the especially disastrous epidemic in the San Joaquin valley of California in 1930, the investigations of Meyer and associates [1] established that this outbreak and probably some earlier ones represented the appearance of a new disease, encephalomyelitis due to a filtrable virus. In the subsequent years this disease or the recognition of it spread gradually eastward. Reports of a similar disease also began to appear from foreign countries, especially Russia.

Naturally, apprehension was soon felt that this virus might presently establish itself in man, especially in farmers and stock men in close contact with the sick horses. However, even during the height of the severe California equine epidemic, there were only a few single cases reported among men working with horses.

The first alarming transfer of the disease from horse to man apparently occurred in southern Massachusetts in the late summer of 1938. In this instance a severe epidemic among the horses of the region preceded the appearance of the first human cases. During August and September 38 human cases were reported,[2] with a mortality of 65 per cent. From 8 of the patients virus of the Eastern type of equine encephalitis was isolated by animal inoculation. A summary of the clinical observations on these patients and a description of the pathologic changes in the nervous system have been published by Wesselhoeft, Smith and Branch.[3] By postmortem examination of 7 persons, from 4

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From the Division of Pathology of the National Institute of Health, United States Public Health Service.

1. Meyer, K. F.: Ann. Int. Med. **6**:645. Howitt, B. F.: J. Infect. Dis. **51**:493, 1932.

2. Fothergill, L. D.: Tr. Am. Neurol. A. **65**:9, 1939.

3. Wesselhoeft, C.; Smith, E. C., and Branch, C. F.: J. A. M. A. **111**: 1735, 1938.

of whom virus was obtained, these investigators determined that the Eastern type of the disease in man consists of severe, acute and widespread encephalomyelitis. Grossly, the brains showed only intense vascular engorgement, generally most pronounced in the pons and the medulla. Microscopically, the characteristic lesions appeared to be focal and diffuse infiltrations mostly by polymorphonuclear leukocytes. Discrete and confluent patches of necrosis of the parenchyma appeared in varying degree. Throughout there were prominent perivascular collars, and frequently the cells were almost entirely polymorphonuclear leukocytes. Often the inflammatory cells spread into the nearby parenchyma. In addition, the walls of some arteries were so markedly infiltrated by leukocytes that the lesion could be classified as acute arteritis. Lesions were distributed irregularly without regard for anatomic or functional units in the cortex, the basal ganglions and the brain stem. Occasional small foci were seen in the molecular layer of the cerebellar cortex and in both anterior and posterior horns of the cord. The nerve cells in the lesions showed all grades of degenerative changes up to necrosis and neuronophagia. No inclusion bodies were observed.

In the meantime, equine encephalomyelitis had been smoldering with sporadic development in man in the Middle and Far West since the severe epidemic in horses in California in 1930. Suddenly and without apparent cause, in the summer of 1941, the disease burst out in acute epidemic form among the farmers in the North Central states. In the first two weeks of July 1941, 35 cases were reported for North Dakota. In the succeeding weeks, the number increased rapidly, and the disease appeared successively in the contiguous portions of South Dakota, Minnesota and southern Manitoba. The occurrence of new cases reached a peak in mid-August. Thereafter they declined till, by mid-September, the epidemic appeared to have run its course. In all over 2,000 cases were reported from this area, and about 10 per cent of the patients died. In the early weeks of the epidemic the mortality rate seemed considerably higher, but later as more mild illnesses were correctly diagnosed, the average death rate fell to about 10 per cent. However, even the first reports, which indicated a death rate of nearly 50 per cent, did not equal the average mortality of 65 per cent reported in the much smaller outbreak of the Eastern type of the disease in Massachusetts three years earlier.

The disease appeared almost exclusively in rural areas. In interesting contrast, the regional cities of St. Paul and Winnipeg were at the same time undergoing moderate epidemics of poliomyelitis, and the cases of poliomyelitis were equally confined to the cities and suburbs. In view of the possible existence of a mosquito vector for encephalomyelitis,[4]

4. Merrill, M. H.; Lacaillade, C. W., and Ten Broeck, C.: Science **80**: 251, 1934.

it is worth noticing that Canadian authorities mentioned that the summer had been unusually hot in Manitoba, with much rain, and that owing to war conditions mosquito control had been less vigorous than usual.

The majority—at least two thirds—of the patients suffering from encephalomyelitis were adult farmers. The remaining patients were women or children under 15. In contrast, a high proportion of children were stricken in the Eastern epidemic of 1938.

CLINICAL NOTE

The clinical course of the disease in fatal cases appears quite uniform. The incubation period of the natural disease has not yet been determined, but Helwig's [5] report of the accidental infection of a laboratory worker suggests that it is in the neighborhood of fourteen days. The onset is abrupt, without apparent prodromal signs, and can usually be timed with considerable accuracy. Generally the illness begins with severe frontal headache, promptly followed by dizziness and prostration. When first examined the patient is usually very ill, disoriented or partly stuporous, and has moderately high fever. The neck is tender and stiff, sometimes retracted. Frank paralyses are seldom observed, but the limbs show some tremor and spasticity, which fluctuates considerably during the course of the disease. The Babinski sign may also be elicited from time to time. The leukocyte count is moderately elevated. In contrast to the severity of the illness, the cell count of the spinal fluid is rarely above 100 cells per cubic millimeter, and about two thirds are lymphoid cells. The pressure is only mildly elevated, the Pandy reaction is weakly positive, and the colloidal gold test may show a weak reaction of the meningitic type. Death usually occurs about the sixth day but may occur as early as forty-eight hours after onset.

MATERIAL AND METHODS

Through the cooperation of Dr. M. M. Williams, of the North Dakota State Health Department, and of Drs. P. J. Breslich and O. R. Kelley in the epidemic area, we received specimens of varying completeness of 13 brains. From 7 of these the virus of the Western type of equine encephalomyelitis had been isolated by animal inoculation at the Rocky Mountain Laboratory of the United States Public Health Service in Hamilton, Mont. All tissues had been fixed in solution of formaldehyde U. S. P. Depending on their completeness, up to 80 large blocks were cut from each specimen according to a uniform scheme and embedded in paraffin. All sections were routinely stained with eosin–methylene blue and completely examined with a mechanical stage. Sections from selected blocks were stained with gallocyanin and by Weil's method to demonstrate myelin sheaths. Frozen sections from other blocks were stained with sudan IV for fat, by various impregnation methods for neuroglia and by the Biel-

5. Helwig, F. C.: J. A. M. A. **115**:291, 1940.

schowsky technic for neurofibrils. The following description of the nature and the distribution of the lesions of equine encephalomyelitis is an average composite picture constructed from the foregoing examinations.

HISTOLOGIC NATURE OF LESIONS

The individual lesions making up this severe encephalitic process are of three general sorts. The first and most widespread is represented by a patch or nodule of proliferating microglia (fig. 1 *A*). Such foci vary from loose aggregations to tightly packed nodules. The component cells show all transition stages from rod cells through forms with bizarre and contorted nuclei to monocytes with well defined cytoplasmic bodies. However, in contrast to the lesions caused by the Eastern strain of virus, only rarely can one or more polymorphonuclear leukocytes be identified among the reacting glial cells. The marked distortion of nuclei in these inflammatory cells should not lead to one's erroneously classifying them as leukocytes. No cells containing peroxidase granules are demonstrable. On the contrary, until the cells become necrotic they are clearly stainable by the impregnation methods specific for microglia (fig. 1 *B*). Occasionally a glial cell may be seen in mitotic division. In the more compact foci no ground substance or neuropil is visible. In the looser aggregations the ground substance usually appears intact. In a few (fig. 2 *A*), however, it seems less dense and contains a moderate number of vacuoles, probably filled with fluid.

In the gray matter, these foci of microgliosis bear no relation to vessels, and only rarely and apparently by accident do they surround a necrotic nerve cell. Figure 2 *B* shows several such cells in the substantia nigra being attacked by mobilized microglia and their liberated pigment being taken up by phagocytes. Commonly, however, seemingly intact nerve cells may be seen in the midst of the microglial foci.

The microglial nodules in the white matter are smaller and less numerous than those in the gray matter. They are generally compact and frequently surround small vessels (fig. 3 *A*). The cytoplasm of the reacting cells is often slightly foamy, but the cells contain little material stainable with sudan. Some demyelination is demonstrable within these cellular foci, but it is considerably less conspicuous than might be expected from the size of the lesion. Evidently these proliferative foci form rapidly, and in fatal cases death occurs before there is time for the production of well marked degenerative changes in the myelin sheaths. In the anatomic diagrams to follow, these foci of microgliosis in the gray and the white substance are indicated by small groups of dots.

The second sort of lesions (fig. 3 *B*) comprises much larger foci of change but these are less numerous and widespread, and are seen clearly only in the gray matter. The typical lesion consists of a sharply defined area of partial solution of the ground substance, leaving a

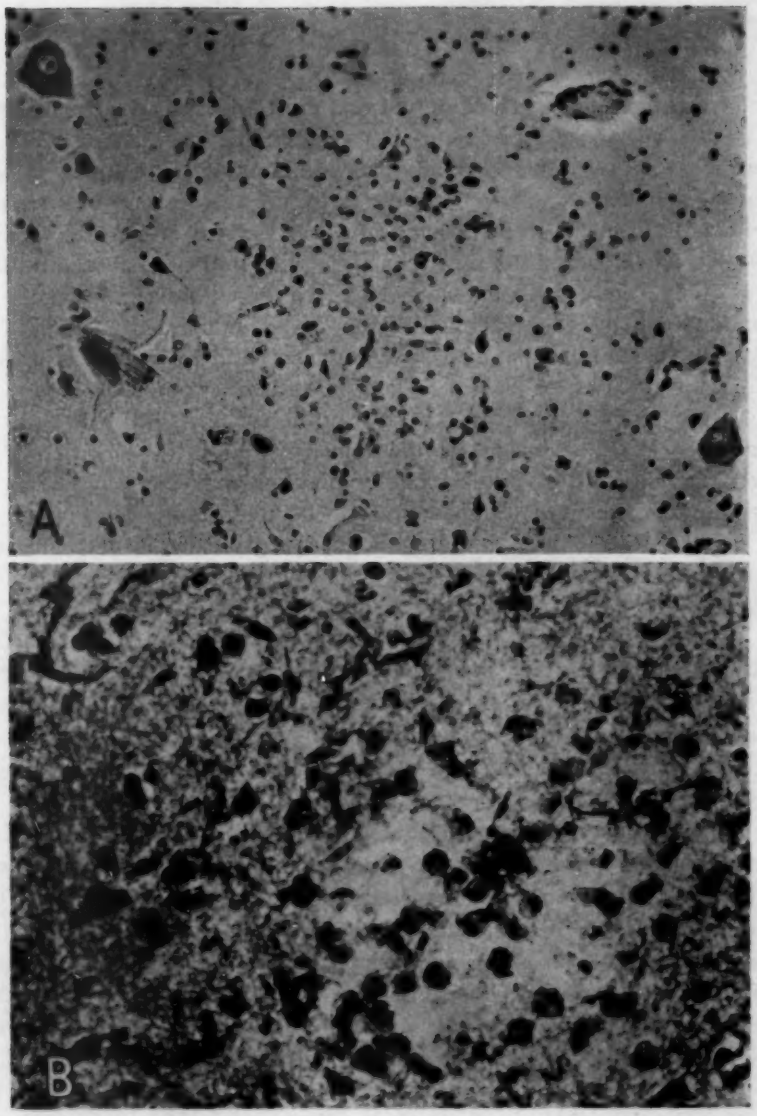


Fig. 1.—*A*, nodular microgliosis in the motor cortex (paracentral lobule). Gallocyanin; × 150. *B*, silver impregnation of a small cortical nodule showing specific staining of the cyptoplasm of the reacting microglia. Penfield II modification for microglia and oligodendroglia; × 350.

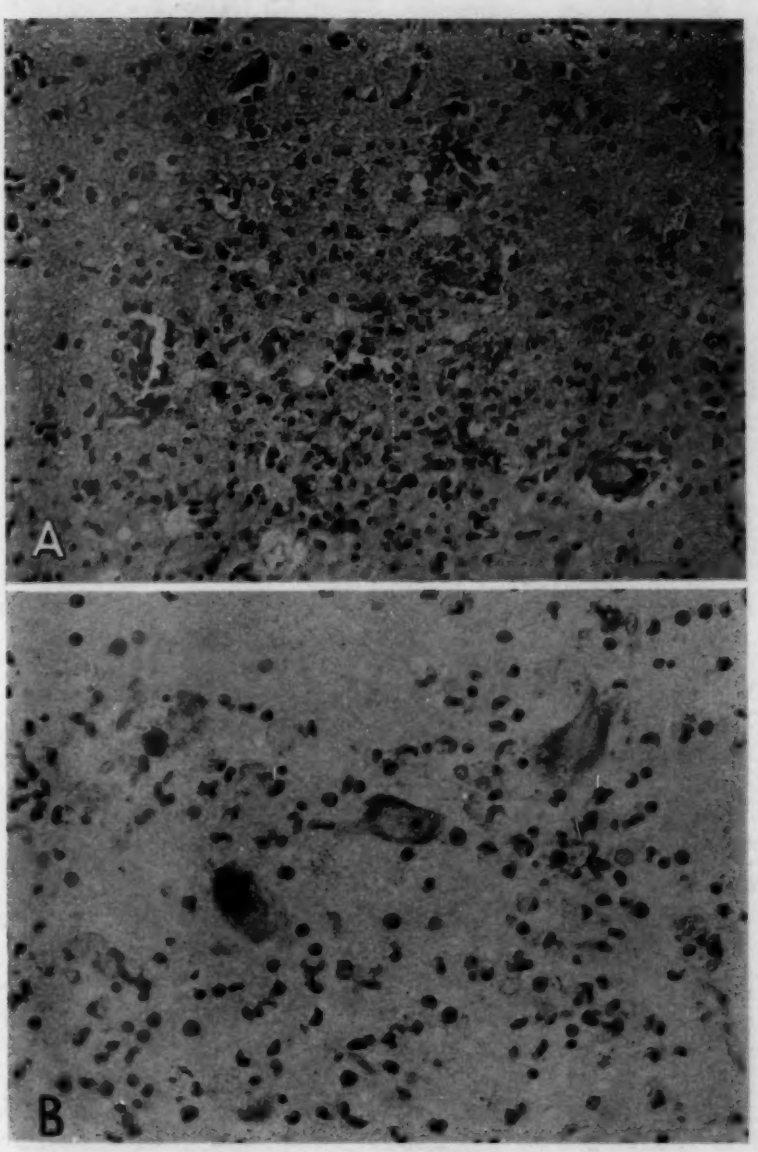


Fig. 2.—*A*, focal microgliosis in the cerebral cortex showing vacuoles in the ground substance. Gallocyanin; × 150. *B*, substantia nigra showing microgliosis, necrosis of nerve cells, neuronophagia and phagocytosis of liberated pigment. Gallocyanin; × 310.

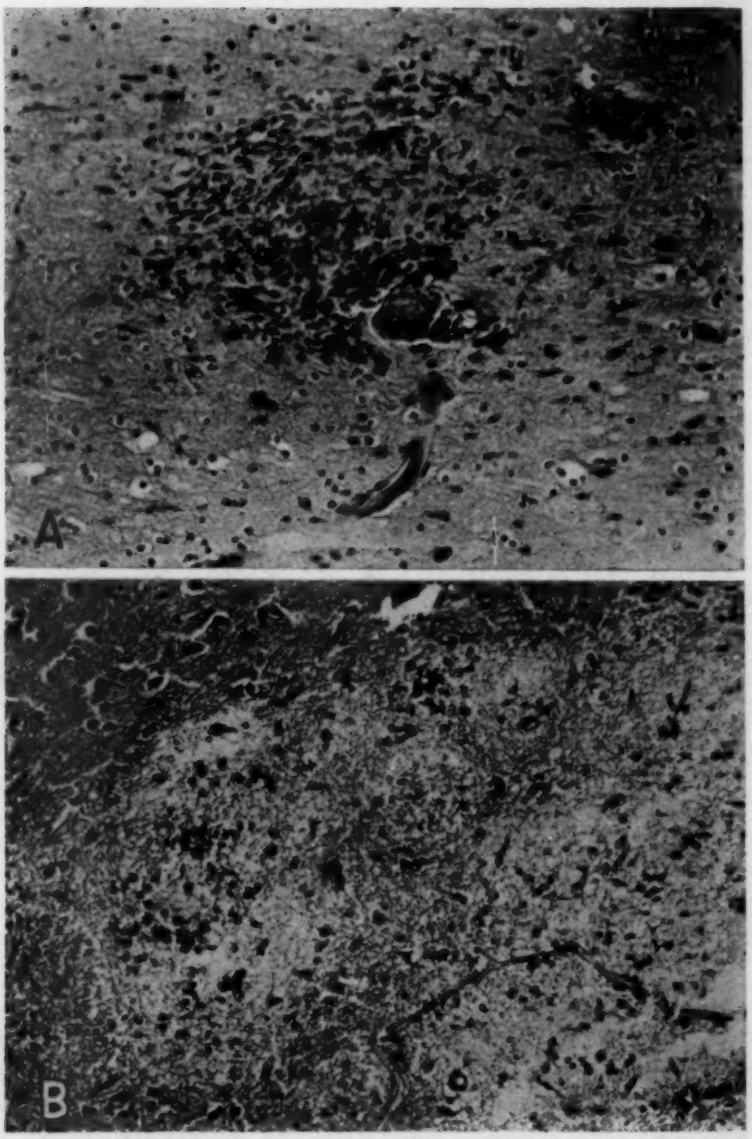


Fig. 3.—*A*, white matter presenting circumvascular microgliosis and partial demyelination. Eosin-methylene blue; × 155. *B*, caudate nucleus with two spongy foci of disintegration of the ground substance and relatively scanty cellular infiltration. Eosin-methylene blue; × 73.

spongy or weblike patch containing relatively few inflammatory cells. The surrounding tissue often appears somewhat compressed, as if such patches were distended by an acute edema. Many of the larger patches are evidently formed by coalescence of several smaller foci. They range in size from microscopic lesions to areas more than 1 mm. in diameter, which are grossly visible as small translucent pits in the cut surface of fixed brain tissue. These lesions are represented by cross-hatched symbols in the subsequent anatomic diagrams.

A higher magnification (fig. 4*A*) of this same lesion reveals that nearly all the inflammatory cells are microglia, though often their nuclei are greatly contorted and pyknotic. A number have phagocytosed bits of nuclear material. Others present cytoplasmic vacuoles apparently containing some sort of fluid, as only rarely are there stainable globules of fat inside them. Within the spongy patches most of the nerve cells have disappeared. Those remaining have undergone coagulation necrosis, but such dead cells do not appear to attract phagocytes, and no neuronophagia is observed. Bielschowsky preparations (fig. 4*B*) reveal an extensive loss of nerve fibers, increasing with the size of the lesion. Many of those still stainable are kinked or broken into fragments of varying length. Within the nerve cells included in the lesion the neurofibrils are frequently agglutinated into one or more coarse bands along the cell wall. No inclusion bodies were observed in any of the specimens examined.

The relation, if any, of these spongy patches to vessels is difficult to determine. In at least half of them, as in the one here illustrated, a small but intact vessel passes through the field. Indeed, it would be hard for occasional small vessels not to be included when lesions of this size are located in the richly vascular gray matter. At any rate, the lesions do not resemble microinfarcts, nor have any occluded vessels been observed in or near them. It also seems improbable that these spongy patches develop from the nodules of microgliosis previously described. Though a number of foci of both sorts may be observed side by side in the same section, lesions of transitional appearance are quite uncommon. Many of the smallest patches seem to develop as spots of acute edema in the ground substance, practically free of any inflammatory cells.

In addition to these two sorts of parenchymatous lesions, there is a varying degree of cellular infiltration about vessels traversing or draining affected areas. The infiltrate is only moderate in amount and is composed almost entirely of lymphoid cells. Among these are occasionally a few mononuclear phagocytes containing hemosiderin, globules of fat or bits of nuclear debris. Only rarely, however, are one or more polymorphonuclear leukocytes seen, generally about small vessels in the vicinity of early lesions. No massive leukocytic infiltration of vessel walls resembling the arteritis described in the Eastern disease was observed in our material.

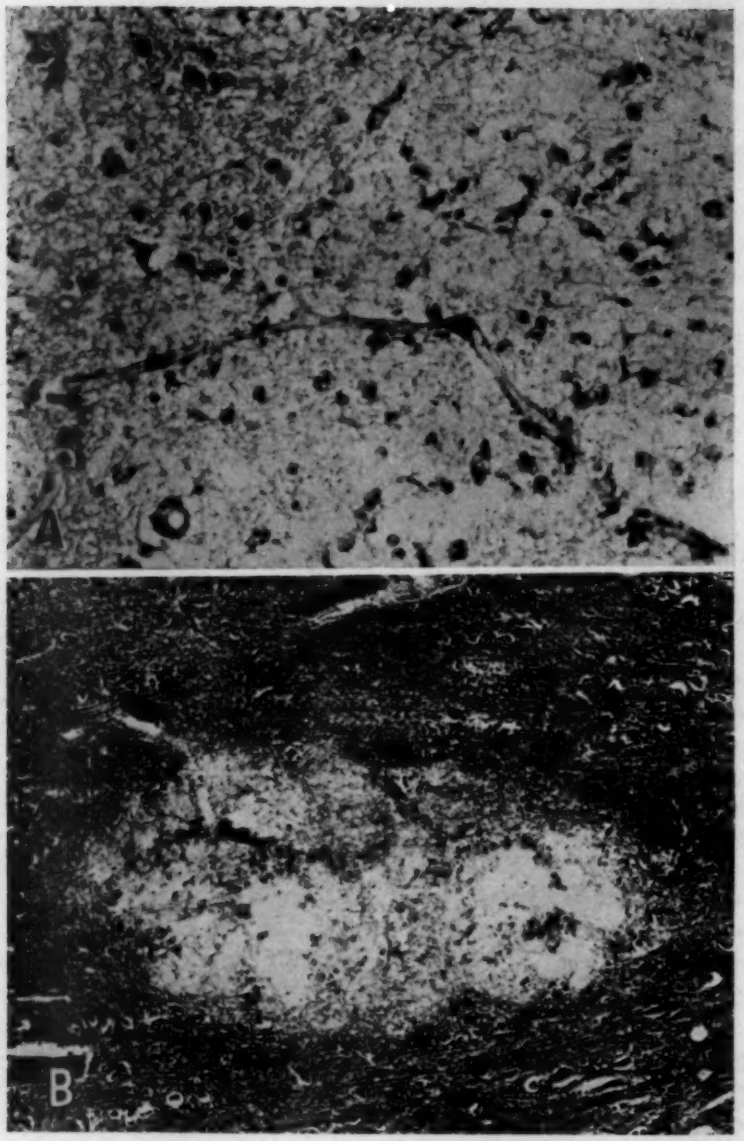


Fig. 4.—*A*, higher magnification of the spongy focus shown in figure 3 *B*. Note the patent capillary traversing the lesion and the character of the inflammatory cells. Eosin-methylene blue; × 120. *B*, small spongy focus in the cortex showing loss of nerve fibers and ground substance. Bielschowsky; × 96.

Meningeal infiltration is scanty, agreeing with the low spinal fluid cell count; the infiltrate consists only of small foci of round cells scattered at random in the subarachnoid space. No acute lesions were observed in the choroid plexus or the ependyma, but degenerative fibrosis was fairly common in the stroma and neuroglial granulations in the ependyma. Such minor changes were not more than might be considered normal for the advanced age of most of our subjects.

ANATOMIC DISTRIBUTION OF LESIONS

Chief attention in this description of equine encephalomyelitis is devoted to the comparatively neglected anatomic pattern of the disease process, rather than to the histologic details. Certainly there is little characteristic about the perivascular collars of round cells since such collars are regularly observed in the neighborhood of many injuries of the brain, regardless of whether they are caused by infection, trauma and degeneration, or neoplasm. The nodules and patches of microglial proliferation occur less commonly and so have greater significance. However, they are seen in a variety of nonbacterial inflammatory lesions, and their diagnostic meaning is largely determined by their number and location. Such also is the case with necrosis of nerve cells, with or without neuronophagia. Even the spongy patches of necrosis and edema in the ground substance are not individually pathognomonic of equine encephalomyelitis. Their number and size vary widely in individual specimens, and occasionally small patches of quite similar appearance may be seen in the medulla in acute bulbar poliomyelitis. In short, we believe that the diagnostic features of the encephalitides, including equine encephalomyelitis, are to be found not so much in the minute structural details of individual lesions as in the pattern of those lesions as determined by a systematic examination of the entire brain.

The anatomic distribution of the disease process is presented in the following diagrams of selected levels of the brain. The same three symbols to represent types of lesions are used in all. Nodules of microgliosis are indicated by groups of dots; spongy patches of necrosis and edema in the ground substance are designated by cross-hatched signs; small circles represent perivascular collars of lymphoid cells.

The cortical lesions vary in different specimens from a few in the dorsal part of the central gyri to innumerable foci spread through the entire cerebral hemispheres. No single region seems to be entirely immune. On the average, however, there is a moderate number of lesions, distributed as shown in figure 5 *A*, chiefly in the dorsal and anterior regions, corresponding roughly to the projection field of the fibers forming the internal capsule.

Lesions also occur in the olfactory bulbs, though our material is not sufficient to determine their frequency. One pair is severely damaged,

one single bulb shows a few focal and perivascular infiltrations, and another single bulb is apparently intact.

The anterior part of the basal ganglions, illustrated in figure 5 *B*, regularly presents the most severe damage. Interestingly enough Hurst [6] showed in experimental animals that, regardless of the route of inoculation, this region shows the first lesions and is the first part of the nervous system to become infective. Lesions are strikingly concentrated in the putamen, the caudate nucleus and the gray stripes joining them across the internal capsule. This localization chiefly in the gray stripes rather than among the fibers proper of the internal capsule may partly explain the inconstant and fluctuating spasticity and Babinski sign exhibited by some patients during their illness. Fewer lesions are present in the anterior perforated substance and amygdaloid nuclei. In most specimens the globus pallidus and the periventricular gray matter are relatively spared, and in some they are practically free of lesions. In the claustrum and the insular cortex damage is moderate and corresponds roughly with the general severity of the disease process in each specimen. In the white matter, chiefly of the corona and the temporal lobes, there are scattered perivascular collars and foci of microgliosis with partial demyelination. Only a few such lesions appear in the corpus callosum and the fornix. No damage was observed in the optic chiasm and tracts in any specimen.

At the level of the middle of the thalamus (fig. 5 *C*) lesions are somewhat less numerous but still tend to concentrate in the body and tail of the caudate nucleus and in the putamen. In fact, numerous foci are found in the putamen as far posteriorly as strands of it are visible in the central white matter. A moderate number of lesions, the majority in the form of foci of microgliosis, are scattered at random in the thalamic nuclei. Again the globus pallidus and the central gray matter show relatively little damage. In some specimens single foci are seen in the subthalamic and mamillary bodies. A few lesions were found in the lateral geniculate bodies of some specimens, though the optic tracts appeared unaltered.

The amount of damage in the hippocampus varies considerably and appears to correspond roughly to the general severity of the disease process in each specimen. In all cases lesions are practically confined to the layer of large pyramidal cells. They range from small nodules of microglia to large patches from which many pyramidal cells have vanished, with the remaining few necrotic and coagulated. Only in the most severe disease are a few small nodular regions of microgliosis to be seen in the small cell layer of the dentate gyrus. In a single specimen there was total coagulation necrosis of the ventrolateral quadrant (Sommer's sector) of one hippocampus, with practically no cellular inflammatory reaction. This sort of lesion has been described in a

6. Hurst, E. W.: J. Path. & Bact. **42**:277, 1936.

variety of conditions in both man and animals. It is probably due to vascular disturbance rather than to virus action, though no occluded vessels were observed.

In the midbrain (fig. 5 *D*) the most severe injury is seen in the substantia nigra. Occasional necrotic cells are present, as well as a number of inflammatory foci (fig. 2 *B*). In one specimen, as indicated on the right in figure 5 *D*, there were several thrombosed vessels and a

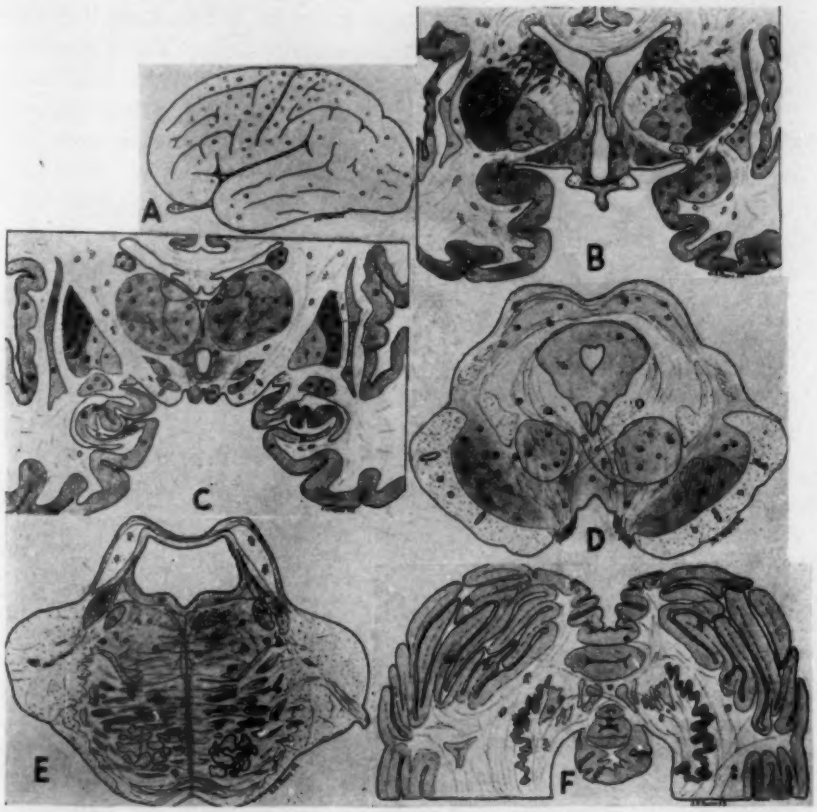


Fig. 5.—*A*, diagram of the cerebral hemisphere showing the average distribution of lesions in the cortex. The same symbols to represent lesions are used in all diagrams, viz., groups of dots for foci of microgliosis, cross-hatched signs for spongy foci and circles for perivascular collars of round cells. *B*, diagram of the basal ganglions at the level of the optic chiasm showing distribution of lesions. *C*, diagram of the midportion of the thalami at the level of the mamillary bodies. *D*, diagram of the midbrain at the level of the anterior colliculi and the roots of the oculomotor nerves. *E*, diagram of the pons at the level of the trigeminal roots. *F*, diagram of the cortex and the roof nuclei of the cerebellum.

large area of hemorrhage and softening. Scattered focal and perivascular infiltrations and rare spongy patches appear in the red nuclei and the anterior colliculi. Sometimes there are single foci in the central gray matter about the aqueduct, but none were seen within the oculomotor nuclei. Vessels traversing the peduncles are frequently surrounded by collars of lymphoid cells, but there is little demyelination evident in the peduncles.

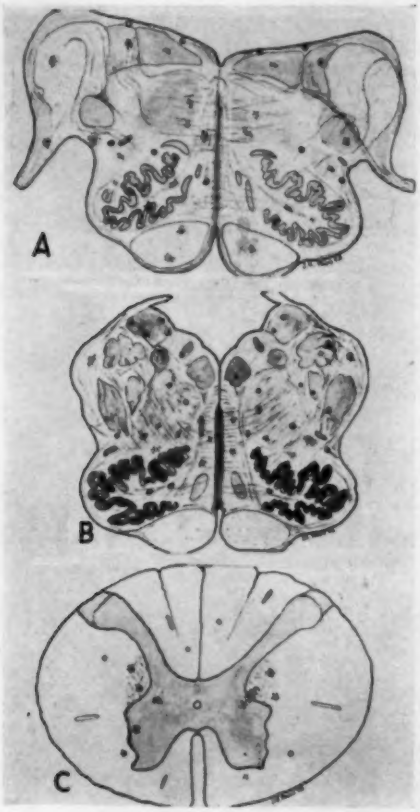


Fig. 6.—*A*, diagram of the anterior part of the medulla through the roots of the acoustic nerves. *B*, diagram of the posterior part of the medulla at the level of the nuclei of the hypoglossal nerves. *C*, diagram of the first cervical segment of the spinal cord.

A section through the pons at the level of the trigeminal roots (fig. 5 *E*) shows a moderate number of lesions spread fairly evenly through both the tegmentum and the base. This is in marked contrast to poliomyelitis, in which the lesions are practically confined to the tegmentum. In the base of the pons most of the inflammatory foci are located in the interfascicular gray matter rather than in the bundles of myelinated nerve fibers. It is also noteworthy that caudal to this

level the spongy patches of disintegration of the ground substance are rarely seen. Instead, the inflammatory reaction consists only of nodules of microgliosis and perivascular collars of lymphoid cells.

In the cortex of the cerebellum (fig. 5 *F*) there are scattered microglial foci in the form of vertical streaks in the molecular layer. Such foci often appear to originate in the Purkinje layer and spread toward the surface, but only exceptionally is a necrotic Purkinje cell seen within them. Larger compact microglial nodules, rarely containing a necrotic nerve cell, are found in the central gray masses. Usually they are more numerous in the dentate than in the tectal nuclei. A few perivascular collars and small patches of microgliosis with partial demyelination appear in the white matter.

A diagram of the anterior part of the medulla (fig. 6 *A*) is presented to show the scarcity of lesions in the reticular substance and vestibular nuclei—regions which are severely damaged in bulbar poliomyelitis. A few large compact foci are seen in the gray matter and the hilus of the inferior olives, and smaller nodules with slight demyelination appear in the pyramids and the restiform bodies.

In the midportion of the medulla (fig. 6 *B*) and in diminishing number caudally, perivascular collars and small microglial foci are sparsely scattered in the gray and the white matter. Their distribution appears entirely casual, without predilection for any particular structure. A few small cellular foci with slight demyelination occur in the restiform bodies, but none were seen in the pyramids at this level.

The upper part of the cervical cord (fig. 6 *C*), the only portion available for study in the material collected, shows only slight infiltration about penetrating vessels and a few microglial foci concentrated in the reticular process and the nearby base of the anterior horns.

COMMENT

It is apparent from a consideration of the specimens here described that the virus of equine encephalomyelitis can cause in man an inflammatory process anatomically more severe and widespread than that observed in any other recognized variety of encephalitis. Only poliomyelitis approaches it in the severity of destruction of nerve parenchyma, but the lesions of the brain in poliomyelitis are maximal in the tegmentum of the medulla and the pons, and in the cortex they are strictly limited to the precentral gyri. In contrast, no single region or structure of the brain parenchyma appears to be entirely resistant to injury by the virus of equine encephalomyelitis. This diffuse pattern of lesions supports the hypothesis, suggested by animal experiments and by the probable transmission by an insect vector, that the virus reaches the brain by way of the blood stream. No evidence suggesting a neuronal path of transmission was observed, and the relative scarcity of lesions in the periventricular tissues makes it unlikely that the spinal fluid plays

any active role in disseminating the virus. However, a considerable degree of tissue susceptibility must operate to produce the definite concentration of lesions in the anterior and dorsal regions of the brain, especially in the caudate nucleus and the putamen. It is also interesting that in spite of the probable infection of the brain via the blood stream the lesions may not be symmetric but one hemisphere may show considerably more damage than the other.

The results of this anatomic investigation do not materially assist in solving the problem of the relationship between the serologically distinct Eastern and Western varieties of equine encephalomyelitis. From published descriptions it seems that the number and the distribution of lesions in the two varieties are about the same. However, investigators working with the Eastern strain emphasize the prominent part played by polymorphonuclear leukocytes in the parenchymatous, vascular and meningeal inflammation and only exceptionally mention patches of partial destruction in the ground substance. In contrast, the lesions in this Western material are composed almost exclusively of mononuclear cells—lymphoid cells and monocytes about vessels and mononuclear cells of microglial origin in the parenchyma. Though such reactive cells are often so contorted as to resemble leukocytes at first sight, leukocytes identified either morphologically or by the peroxidase reaction are scarce.

SUMMARY

Thirteen brain specimens from the 1941 epidemic of equine encephalomyelitis in North Dakota have been subjected to detailed examination, and the histologic nature and the anatomic distribution of the lesions in them have been described and illustrated. The Western strain of the virus of equine encephalomyelitis had been isolated from 7 of them.

A summary of the findings shows that the Western virus can produce in man fatal encephalitis characterized by nodules of microgliosis, spongy areas of partial destruction of ground substance and perivascular round cell infiltration. The great majority of the inflammatory cells are mononuclear, in contrast to the predominance of polymorphonuclear leukocytes described in the Eastern type of equine encephalomyelitis. The lesions regularly appear in greatest number and severity in the anterior parts of the putamen and the caudate nuclei. In the brain stem the lesions are almost entirely proliferative rather than destructive and are distributed fairly evenly in the tegmentum and base, independent of anatomic and functional units. The number of lesions diminishes markedly in more caudal levels, and only a few small foci appear in the upper part of the cervical cord. A variable number of lesions are also present in the cerebral cortex, chiefly in the anterior portion of it, and in the cortex and the roof nuclei of the cerebellum.

Case Reports

ACUTE PANCREATITIS FOLLOWING BLOOD TRANSFUSION

LAUREN V. ACKERMAN, M.D., COLUMBIA, MO.

A search of the literature fails to reveal any report of acute hemorrhagic pancreatitis following transfusion of incompatible blood.

REPORT OF A CASE

An 83 year old white man was admitted to the hospital for treatment of a tumor beneath the right mandible, diagnosed as melanocarcinoma. The tumor had recently shown increased growth and pigmentation. The patient was in excellent general health, well developed and well nourished. On the right side of the neck, just beneath the midportion of the mandible, was an irregular brown discoloration, 3.5 by 2 cm., with a central ulcer, 1.5 by 1.6 by 0.2 cm. The heart was slightly enlarged, and the blood pressure was 220 systolic and 80 diastolic. The urine was essentially normal, and the blood count was normal. The Kahn test was negative.

Following preoperative administration of soluble phenobarbital, morphine and atropine, with the patient under local anesthesia, the lesion was excised by radical dissection of the right side of the neck. The procedure took two hours and fifty minutes. The patient's condition throughout the operation was satisfactory, the blood pressure varying from 220 systolic and 80 diastolic at the start to 200 systolic and 80 diastolic. Because of his age and the duration of the operation, a transfusion seemed advisable. The patient's blood was type O; through error, blood of type A was used. At the end of thirty minutes, after approximately 200 cc. had been transfused, the patient suffered a severe chill, marked decrease in blood pressure and other signs of shock.

Bleeding into the wound in the neck was noted, and on the supposition that some large vessel had broken loose from its ligature, the wound was explored. No single large bleeding point was found, but there were a dozen punctate areas of oozing, which were ligated. The patient continued in shock, and oxygen therapy and intravenous infusions of dextrose and saline solution, as well as a transfusion of 500 cc. of compatible blood, were given. There was no reaction from this transfusion. The blood pressure began to fluctuate through rather wide margins but never reached more than 160 systolic. Approximately ten hours after the transfusion of incompatible blood, the patient complained of pain in the lower part of the abdomen. Examination revealed no evidence of spasm. The blood pressure, after the appearance of this symptom, was never higher than 60 systolic, and the diastolic pressure could not be obtained. He continued in shock, and all measures to combat the condition were of no avail. He died approximately twenty-five hours after the first transfusion.

Autopsy.—The anatomic diagnoses were: thrombosis of pancreatic veins and branches of the right colic veins; acute hemorrhagic pancreatitis; "transfusion" kidneys; chronic pyelonephritis; acute ulceration of the ascending colon; mediastinal emphysema; cardiac hypertrophy, and bilateral hydrothorax.

From the Ellis Fischel State Cancer Hospital.

The body was fairly well developed. There were several recent surgical incisions in the neck, but no evidence of melanoma. The peritoneal cavity contained about 150 cc. of cloudy blood-tinged fluid, and there was diffuse recent peritonitis. Approximately 300 cc. of thin cloudy fluid was present in each pleural space, but no petechial hemorrhages were visible on the pleural surfaces. The lungs were slightly emphysematous and somewhat congested. Moderate mediastinal emphysema was present. The heart weighed 470 Gm.

The first two thirds of the pancreas showed patchy areas of necrosis, which gave it a somewhat splotchy yellow appearance. It also looked hemorrhagic, and section showed apparently recent thrombi distending the pancreatic veins. There were no thrombi in the tail, which appeared normal. The liver showed moderate congestion. The gallbladder was grossly normal. The main pancreatic and the common bile duct had separate entrances into the duodenum.

The right kidney weighed 220 Gm. and the left 60 Gm. The vessels to both kidneys were normal. The capsule of the right kidney stripped with ease, and on section the cortex was about 7 mm. in thickness. The pyramids showed rather prominent congestion. The left kidney revealed chronic pyelonephritis. The capsule stripped with difficulty, taking with it small bits of grayish red parenchyma. On section, the cortex was irregular, narrowed and merged with a distorted medulla. Both ureters were patent and normal.

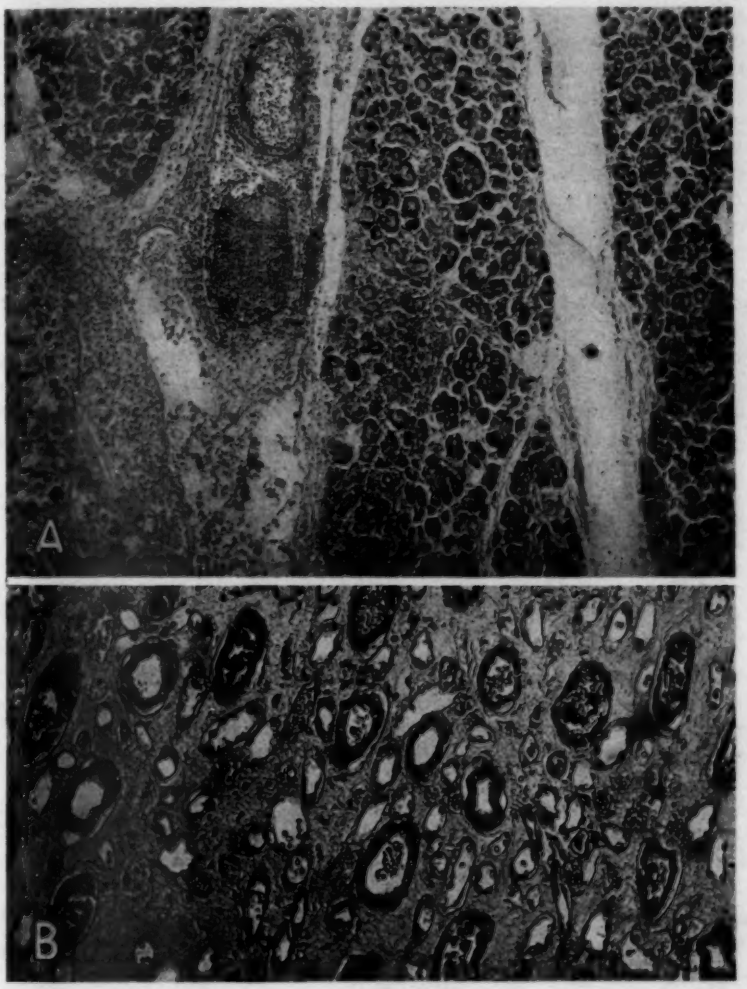
In the ascending colon just above the cecum, there was a small, rather poorly demarcated area of superficial ulceration, measuring about 3 by 3 cm. Section showed a few recently thrombosed veins.

Microscopic Examination.—The heart presented marked fragmentation of the myocardial fibrils. The capillaries of the alveolar walls of the lungs were markedly dilated and filled with red blood cells. There was slight edema of the alveolar spaces. The spleen showed marked hyperemia, together with large collections of mononuclears filled with brown pigment. Some of this pigment was free.

Both the large and the small veins of the pancreas showed multiple recent thrombi, in which free yellow pigment was seen. In some veins there was destruction of the wall with migration of red blood cells into the parenchyma. Large collections of mononuclears were filled with brown pigment. Patches of destruction of pancreatic tissue were present, in which the cells of the acini took the stain poorly and there was an accompanying fibrinous exudate with polymorphonuclears. In some areas, thrombi were seen in the veins, surrounded by normal-appearing pancreatic tissue. Multiple sections failed to reveal changes in the arteries or the pancreatic ducts, nor was there evidence of chronic pancreatitis.

The stomach showed some congestion but no thrombosis of the vessels. The congestion was present predominantly in the capillaries and venules of the villi. A section from the ascending colon showed ulceration of the mucosa and an acute exudate, with recent thrombosis of moderate-sized veins. The liver disclosed slight central hyperemia but no necrosis. Both kidneys presented the typical changes from transfusion of incompatible blood. In the capsular spaces of the glomeruli were spherical masses of eosinophilic amorphous material. The same type of debris was present within the convoluted tubules, and the cytoplasm of the lining cells was granular and the nuclear staining poor. These changes were somewhat patchy. In the loops of Henle and in the distal convoluted tubules there were a few aggregations of broken-down blood pigment. Numerous hemoglobin casts were present throughout the collecting tubules. There was little change in the interstitial tissue except for slight edema without cellular infiltration. All of the capillaries and venules were hyperemic, and in a few of them washed-out red

blood corpuscles could be seen. The kidney, weighing 60 Gm., showed typical chronic pyelonephritis, with some changes in the arterioles. The relation of the patient's hypertension to these changes will not be discussed. The other organs were not remarkable except for widespread dilatation and hyperemia of small venules and capillaries.



A, pancreas showing thrombosis exudate and necrosis; × 60. *B*, kidney showing hemoglobin casts within collecting tubules and interstitial edema; × 60.

COMMENT

The symptoms due to renal insufficiency usually dominate the picture in fatal blood transfusion. Death from uremia occurs in from four to ten days. In many reports of cases, the kidneys were the only organs described. The uremia has been explained on the basis of

mechanical obstruction due to the breakdown of hemoglobin with formation of casts in an acid urine. However, the number of tubules involved does not appear to explain the uremia. DeGowin and coworkers [1] emphasized the necrotic, poorly staining tubular epithelium and the marked interstitial edema. They expressed the belief that these changes are important but did not explain them. Anderson [2] suggested that some of the renal damage is on a vascular basis. In the cases reported by Bywaters [3] of death due to traumatic anuria, the renal changes closely simulated those found in fatal blood transfusion. In our case there was widespread dilatation of the renal capillaries, and in a few capillaries washed-out red blood cells and aggregations of pigment were seen. These changes, together with the probable damage of the capillary endothelium, must have interfered with oxygenation, which in turn interfered with the nutrition of the renal epithelium. This would adequately explain the tubular damages described. The persistent low blood pressure would also interfere with renal function.

After the kidney, the liver has most frequently shown alteration. Hepatic necrosis usually is central in origin but may involve any portion of the lobule. Pearce [4] showed that necrosis of the liver may be produced by serum with hemagglutinative power. In his cases the capillaries were actually occluded by fused red cell thrombi, and it is not unlikely that the mechanism may be similar in deaths due to transfusions. In our case these changes were not observed, but in many cases hepatic necrosis has been reported.[5]

Bloody diarrhea and ulceration of the large bowel have also been observed at autopsy [6], but no adequate explanation of this ulceration or description of the regional vessels has been given. In our case, thrombosis seems clearly to have been the cause of the ulceration. It is possible that if a search had been made in other cases, vascular changes might have been noted.

The earliest reference to vascular changes in the lung is that in a report by Kuczynski,[7] whose patient died two hours after receiving a transfusion. The capillaries and the small vessel of the lungs were plugged with recent thrombi. Although there were no thrombi in the vessels in our case, there was marked distention of the capillaries with red blood cells.

Transudate in both pleural cavities has been reported by others,[8] and this finding, together with edema of the lung, ecchymoses, petechiae and general capillary atony, is of course common in shock.

There has been no report similar to ours of changes in the pancreas. The hemorrhagic pancreatitis was doubtless secondary to thrombosis

1. DeGowin, E. L.; Warner, E. D., and Randall, W. L.: Arch. Int. Med. **61**:609, 1938.

2. Anderson, W. A. D.: Personal communication to the author, 1942.

3. Bywaters, G. L.: J. Path. & Bact. **54**:111, 1942.

4. Pearce, R. M.: J. M. Research **12**:329, 1904.

5. (*a*) Lindau, A.: Acta path. et microbiol. Scandinav. **5**:382, 1928. (*b*) Johnson, R. A., and Conway, J. F.: Am. J. Obst. & Gynec. **26**:255, 1933. (*c*) Lemke, R.: Virchows Arch. f. path. Anat. **257**:415, 1925.

6. Jervell, F. H.: Acta path. et microbiol. Scandinav. **1**:201, 1924. Lindau.[5a]

7. Kuczynski, M. H.: München. med. Wchnschr. **65**:485, 1918.

8. Lindau.[5a] Lemke.[5c]

The patient had no previous symptoms to suggest previous attacks of pancreatitis, nor were there any evidences of chronic pancreatitis. The gallbladder was normal, and the common bile duct and the main pancreatic duct entered separately into the duodenum. The pancreatic ducts and the arteries of the pancreas showed no alterations. The pancreatitis appears to have been no more than twenty-five and possibly only fifteen hours old if one times its onset with the complaint of abdominal pain.

The sequence of events in the case might be reconstructed as follows: The transfusion of incompatible blood resulted in shock; shock produced atony of the capillaries, plasma transudation, hemoconcentration and slowed circulation in the areas of venocapillary dilatation.[9] Slowed circulation, hemoconcentration and damaged vascular endothelium favored thrombosis. Large masses of agglutinated red blood cells and free blood pigment created a favorable foundation for thrombosis in the pancreatic and colic veins. The thrombi caused ulceration of the large bowel and hemorrhagic necrosis of the pancreas, which in turn produced further shock. Typical changes were present in the kidney, with many casts filling large numbers of collecting tubules. There was marked damage to the tubular epithelium, and it is probable that if the patient had lived longer, he would have died of uremia due to renal failure.

SUMMARY

A case of acute hemorrhagic pancreatitis following transfusion of incompatible blood is reported.

It is suggested that some of the pathologic alterations previously described in fatal blood transfusion may have been due to shock.

Small and large thrombi probably develop frequently in fatal blood transfusion, with consecutive changes in the corresponding tissues.

9. Moon, V. H.: Am. J. M. Sc. **203**:1, 1942.

General Reviews

EFFECTS OF RADIATION ON NORMAL TISSUES

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VI. EFFECTS OF RADIATION ON THE CARDIOVASCULAR SYSTEM

THE HEART

Relatively little attention has been paid to the effects of radiation on the heart. This organ is so located that there is but slight chance of appreciable doses reaching it unless the radiation is directed to a tumor involving the left mammary or thoracic region, the lower mediastinum or the esophagus. As cardiac muscle is not particularly sensitive, fairly heavy doses of radiation may be absorbed without perceptible effects other than those produced by vascular changes and changes in the interstitial connective tissue.

A fair number of experiments have been aimed at ascertaining the effect of radiation on the contractility of the heart. Desjardins reviewed the early, rather inconclusive experiments on the hearts of cold-blooded animals. Toyoma reported slowing of the contraction rate and lessening contraction of the heart. These changes developed thirty to forty-five seconds after the commencement of treatment with roentgen rays generated at 150 to 170 kilovolts, continued for the duration of the treatment (which lasted only a few minutes) and then gave way to the normal rhythm.

Zwaardemaker and Feenstra suggested that the effect of potassium in inducing contraction of the frog's heart was related to its radioactivity. He found that an isolated, perfused heart which had ceased contraction as a result of lack of calcium could be stimulated to resume contraction by two five minute periods of irradiation with Grenz rays generated at 9,000 volts and 10 milliamperes. This report, however, is not convincingly presented. Oana, using isolated hearts of toads, obtained no effect from roentgen rays, radium or ultraviolet rays.

It has been claimed that electrocardiographic changes and arrhythmia may occur in man following treatment with radiation. However, some of the reports (for instance, that of Parade) were based on observations of hearts already diseased, and others were made in cases in which the radiation was not directed to the heart or in which the ages of the patients were such that definite abnormalities would be expected (Gendreau).

Electrocardiographic changes have been produced in rabbits by roentgen radiation (Gabriel). Warren and Whipple, after irradiating the thoracic and abdominal organs of dogs with roentgen rays, found no clinical or pathologic evidence of changes in the heart and concluded that the thoracic organs were relatively resistant. On the other hand, heavy irradiation of the cardiac region in dogs has produced severe changes even to the point of necrosis and hemorrhage (Davis).

Warthin and Pohle (1929) noted Zenker's degeneration of the myocardium, fatty degeneration of the myocardium, pyknosis of the nuclei of the endothelial cells and chromatolysis of adjacent ganglion cells in the hearts of irradiated rats and rabbits. However, somewhat similar abnormalities were found in some of the control animals. The tolerance dose for the rat's myocardium is said to be 500 roentgens (r) at 140 kilovolts if one accepts Zenker's degeneration of the myocardium, increase of stromal nuclei and small foci of lymphocytes as evidence of damage (Zwaardemaker and Feenstra).

However, the rat's myocardium is extremely resistant. When a dose of 10,000 r (56 r per minute at 200 kilovolts) is given to the precordial region in a single treatment, the myocardium after eight or more days is dark and flabby, with edema, congestion, round cell infiltration, indefinite striations and nuclear swelling or pyknosis. The pericardium and the endocardium appear normal (Leach and Sugiura).

There are a fair number of reports of cardiac damage in man, mostly of a nonspecific character. Granzow observed severe myocardial damage in a 39 year old woman following roentgen treatment. Thibaudeau and Mattick reported 10 cases in which there were changes in the heart ranging from slight interstitial fibrosis to hyaline and fatty degeneration of the myocardium and necrosis. In some of their cases there was round cell infiltration. The patients died from two days to a year after exposure to radiation. The authors do not regard as specific the changes which they encountered. Engelmann also reported nonspecific changes following the use of roentgen radiation, among which granular and vascular degeneration of the myocardium and brown atrophy were noted.

Hartman and co-workers stressed in their 3 human cases the hyalinization and the granular and vacuolar degeneration of the myocardium. Lucarelli mentioned slight degenerative changes in the myocardium following irradiation of the hearts of human beings with roentgen rays at 180 kilovolts.

On the basis of 17 cases in which radiation was directed to the region of the heart, Emery Jr. and Gordon concluded that no damage was done to the myocardium; autopsies were made in 8 cases and electrocardiographic studies in 9.

The application of 30 mg. of radium to the cardiac portion of the stomach produced a perforation of the pericardium 3 cm. in diameter with pneumopericardium (Gottesman and Bendick).

The reports of Bartsch and Wachner on electrocardiographic changes are of little value since radiation was given not to the region of the heart but to the larynx and the upper part of the esophagus, and the changes reported could be, and in view of the age of the patients probably were, the effects of coronary or myocardial disease unrelated to radiation.

The sequence of development of cardiac injuries has been studied in some detail. Warthin and Pohle (1931) gave 500 r at 140 kilovolts as a threshold dose to rats. No clinical symptoms were obtained even at 1,000 r, but after 500 r Zenker's degeneration of the myocardium, increase in stromal nuclei and small focal infiltrations of lymphocytes appeared.

Karlin and Mogilhitsky produced cardiac damage in dogs with from 1 to 3 erythema doses of roentgen radiation. This was evidenced first by perivascular bleeding, second by perivascular swelling with some hyalinization of connective tissue and atrophy of muscle fibers and finally by increased hyalinization of pericardial connective tissue. In some of the nerve fibers myelin degeneration was noted, but no change was detected in ganglion cells.

Ajisaka produced slight vascular congestion in the hearts of rabbits by delivering to the thorax 600 r at 140 kilovolts. After delivery of 3,000 to 5,000 r there were slight histologic changes. In mature rabbits given 9,000 to 10,000 r he found granulation and vacuolation of the myocardium, marked atrophy, pyknosis and degeneration of nuclei, whereas in rabbits 3 days old 300 r caused degenerative changes and 6,000 to 7,000 r caused extensive vacuolation of the myocardium even to complete loss of sarcoplasm. At and beyond the age of 1 month the animals were more resistant. He also attempted to irradiate the hearts of fetal rabbits, giving 600 r to the mother's abdomen. Up to the twentieth day this amount killed all the fetuses; beyond the twentieth day more than 600 r was required to kill all the fetuses. While degenerative changes in the fetal myocardium were observed with this dosage, it is questionable whether the changes were due directly to irradiation of the myocardium or whether they may not have been secondary to the death of the embryos.

Hartman and associates found some clinical evidences of cardiac disturbances in dogs. In some of the animals these were inversion of the T wave and auricular fibrillation and flutter. At autopsy the hearts showed edema of the myocardium, vascular thickening with perivascular mononuclear and polymorphonuclear infiltration, and hyalinization of the connective tissue of the epicardium. The animals had received 5 to 19 erythema doses, and most of them died within thirty days. In irradiated sheep pericardial effusion was noted.

In tissue culture 500,000 to 700,000 r at 35 kilovolts, lightly filtered, was required to cause prompt complete cessation of contractility of embryonic cardiac muscle (Gordon and co-workers).

Tsuzuki found slight fatty degeneration of the myocardium as the only evidence of injury to the heart, although other viscera showed marked changes, when the entire body of a rabbit was irradiated.

Levy and Golden irradiated at 200 kilovolts the hearts of a group of patients who had rheumatic fever, 1 of whom, a 17 year old girl, died a year and four months after the final treatment. She had received roughly 540 r to the heart in nine treatments over a period of five months. There was no change that could be ascribed to the radiation.

On the basis of 17 cases in which radiation had been applied over the thoracic cavity for various cancerous processes, Emery and Gordon decided that there were no demonstrable effects ascribable to roentgen rays. Freid and Goldberg reported the case of a 51 year old woman with lymphoma who received a total dose of 8,370 r during three years. The heart showed marked thickness of the pericardial layers, hypertrophy and interstitial fibrosis of the right ventricle and hyalinization. There was hypertrophy of the fibers of the right auricle. The left ventricle was normal. There was also pneumonitis; so the failure of the right side of the heart which caused death might have been related to that.

Gordon and co-workers irradiated 23 rabbits to varying extents and demonstrated no characteristic changes in the myocardium. Schweizer, on the basis of the case of a 32 year old woman receiving fairly heavy radiation over the thorax during a fourteen month period, regarded swelling and destruction of the sarcolemma and multiplication of the nuclei as characteristic. He noted that the most marked change in the right ventricle was the presence of globules in the myoplasm. The heart was small and soft.

Intense local application of radiant energy will, of course, cause profound damage. In the case reported by Ross a woman was treated by implantation of radium needles for carcinoma of the left breast. One of the 2 mg. needles was lost at the time of removal. Four and a half months later dyspnea developed, and three years later the woman died. At autopsy the needle was found at the interventricular septum with the point in the right ventricle. The pericardium was obliterated by dense fibrous adhesions and for about three fourths of an inch surrounding the needle there was necrotic acellular hyaline connective tissue. About this zone the musculature was replaced by firm, tough glossy material, faintly fibrillar on microscopic examination. The arterioles had disappeared or were occluded. Peripherally there occurred some necrosis of muscular fibers with hemorrhage and some degeneration of capillaries.

Renfer reported as a result of radium treatment for carcinoma of the esophagus localized myocardial degeneration and necrosis with hyaline thickening of the pericardium in 2 cases, both fatal. In 1 case the adjacent endocardium was also thickened.

Faust mentioned pericardial fibrosis as a result of roentgen irradiation of a mediastinal teratoma.

One case of pneumopericardium as a result of radium treatment for carcinoma of the stomach has been reported, but no histologic details are given (Gottesman and Bendick).

Radon in glass inserted in the region of the sinoauricular node in 14 dogs for periods ranging from eight hours to eighteen months (0.6 to 5 millicuries) caused cardiorrhexis and loss of chromatin of the sinoauricular node and adjacent tissue, followed by necrosis, fatty change, connective tissue replacement and calcification. Borman expressed the belief that these changes represented a primary effect on the cells rather than an effect due to circulatory disturbances. The vessels were engorged; later there was rupture of the wall with hemorrhage into the regional tissues. The elastic coats of the larger vessels were very resistant to radiation effect.

In radium poisoning the heart shows little change, as it picks up but little of the radioactive substances. Krebs, in giving the concentrations of radioactive substance in the various viscera of a laboratory worker who had inhaled radioactive material a week before death, found that the heart contained 0.2 per cent, taking the amount absorbed by the spinal marrow as 100 per cent. In the heart of another person, a woman who had been treated by intravenous injections of radium for carcinoma of the cervix, there was 0.5 per cent, and in that of another, who had drunk radium water, there was 0.04 per cent.

The various forms of cardiac damage secondary to radiation therapy cannot be recognized as specific in themselves, but the aseptic necrosis, hyaline fibrosis and obliterative vascular changes combine to form a fairly characteristic lesion.

THE BLOOD VESSELS

One of the most striking clinical effects of radiation is the change induced in cutaneous blood vessels, first apparent as erythema. This erythema, best seen within two weeks after exposure to radiation, has long been a standard for roentgen ray dosage (Failla and co-workers). Months or years later the red tracery of telangiectatic superficial vessels in atrophic skin becomes almost specific evidence of past exposure to radiation. Telangiectasis occurs in about half the cases in which epidermitis has been produced (Cade, page 145).

The early observations [1] established that although no element of the blood vessel is immune to radiation injury, the endothelium is the most susceptible; [2] consequently, the major changes are seen in those vessels in which the endothelium makes up a proportionately large part of the wall. Injury to large vessels is rare with doses below 500 r (Ellinger).

1. Baermann and Linser. Cade (page 145). Gassmann.
2. Bagg. Gudernatsch and Bagg. Rudis.-Jicinsky.

Many references have been made to vascular changes in the various organs and tissues in other parts of this review, and in order to avoid redundancy, only a few will be repeated here.

As early as 1899 the intimal thickening, the swelling and proliferation of the endothelium and the vacuolation of the smooth muscle were noted by Gassmann, who stated these alterations would lead to "starvation of the surrounding tissues" and hence explained the intractable character of the roentgen ulcer. He later (1904) treated rabbits with roentgen rays and sectioned the resulting cutaneous ulcers one month after their development. He found obliteration of lymphatics by endothelial proliferation as well as endothelial proliferation in the arteries and vacuolation of their smooth muscle cells.

In human skin treated with roentgen rays of low voltage, Linser noted at the end of four days fissuring of the media of vessels, occlusion of vessels by thrombi and slight perivascular round cell infiltration, reaching its peak at eight days. After twenty days, intimal thickening by connective tissue was marked, with obliteration of some vessels. During these vascular alterations the cutaneous epithelium showed only slightly increased pigmentation and the hair follicles and glands were normal.

The death of radiation-treated tumor tissue was soon recognized as partly secondary to vascular lesions, among which intimal degeneration and thrombosis were important (Baermann and Linser).

Very early telangiectasia was recognized as a sequel to cutaneous exposure to radiation, sometimes appearing after light doses and almost constantly as a late result after moderate to heavy doses. Unna, in a clinical report on roentgen ray dermatitis, discussed the development of telangiectasia at some length, ascribing it to deep obliterating endophlebitis. This origin has not been clearly proved. There are a number of excellent descriptions of the telangiectases due to radiation.[3] The dilated vessels may be fine or coarse, punctate or weblike, straight or serpentine. Their bright red color appears to shine through the epithelium. They do not disappear spontaneously, although scattered thromboses may occur. While occasionally telangiectases may develop a few weeks or many years after irradiation of the skin, they commonly appear within a year to eighteen months. Those induced by roentgen radiation are indistinguishable from those following radium treatment. They develop from capillaries existing at the time of treatment (Wolbach).

Flaskamp discussed the production of the early erythema of the skin and illustrates clearly the progressive dilatation of the capillary loops.

This dilatation is frequently a reversible change, but with the heavier doses it may become irreversible. With doses under 500 to 700 r delivered at approximately 200 kilovolts, such changes as occur in the capillaries usually are reversible. Interestingly enough, there is evidence,

3. Fabry. MacKee. Porter. Porter and White. Wetterer.

in part at least, that this vasodilatation may actually develop and clear up while a divided dose of roentgen radiation is still being delivered. The period of reversible dilatation lasts for several days or weeks after the initial erythema. During this time dilator stimuli produce a greater effect on the vessels than do constrictor stimuli of comparable strength (Lazarew and Lazarewa).

It has been claimed that the capillary injury is not primarily in endothelial cells but in the nerves supplying them (David and Gabriel). This view has not been generally accepted and is not borne out by our own observations. Ricker, treating the ears of rabbits with mesothorium, interpreted the resultant dilatation as due to greater sensitivity of the dilator fibers of the vascular sympathetic nerves.

There is some evidence that this initial capillary dilatation does not result directly from the effect of radiation on the vessels or on the nerves controlling them but rather through the formation of histamine-like substances. The presence of these in demonstrable amounts in fresh blood has been claimed by Cramer. However, these substances are not of significance in the production of permanent damage to vessel walls.

Stafford Warren and Downing, Bishop and Warren made detailed studies of the response of the stromal blood vessels of the Brown-Pearce rabbit tumor irradiated with 10,000 r. Gross erythema appeared five days after treatment due to damage of the blood vessels, and a considerable part of the redness of the vessels was due to extravasation of large numbers of red blood cells into the perivascular spaces.

The importance of vascular changes in the cutaneous radiation effects was clearly presented by Wolbach. He gave detailed descriptions of the walls of blood vessels in the later radiation changes and defined the changes occurring in the endothelium and the supporting tissues of the walls. If the endothelium was not killed, proliferation often recurred, even to the point of obliteration of the capillaries. Sometimes the swollen or vacuolated endothelial cells formed tufts projecting into the lumen. In the veins and arteries subintimal fibrosis, with the collagen often showing some degree of hyalinization, resulted in thickening of the wall at the expense of the lumen. In the media the elastic tissue degenerated and the smooth muscle cells showed vacuolation, hyalinization or atrophy. This coat was thickened as well by the presence of large, sometimes branching fibroblasts with abundant collagen. The degeneration of elastic lamellas was sometimes complete with substitution of fibrous tissue or bands of hyalinized collagen. He also emphasized the progressive character of the vascular lesions and cited the proliferation of fibroblasts in the media of arteries as late as four years after the last exposure to roentgen rays. However, even in severe damage some normal blood vessels may be seen.

The vascular damage is of fundamental importance because of the resultant lowering of the resistance and reparative powers of all the

tissues, the blood supply of which has been thus impaired (Wolbach). Daland has called attention to the danger of ligation of any vessel in an irradiated field, as the blood supply is already inadequate and further reduction will lead to gangrene.

One of the extensive studies is that of Dobrovolskaia-Zavadskaia. The initial effect is vasodilatation followed by degenerative and reparative changes. With doses up to 1,200 r, complete recovery may occur. With increase above this amount, alteration of the various coats occurs, leading to endothelial proliferation, degeneration of elastica and replacement of smooth muscle with connective tissue. As a rule, the smaller capillaries do not recover if the dose of radiation has been sufficient to produce acute radioepidermitis. The larger the vessel, the greater is the chance for repair and maintenance of patency.

Less attention has been paid to changes in the veins than to those in the arteries (Windholz). Owing to the thinner walls of the veins, phlebosclerosis may be more marked than the fibrotic changes in an artery of similar size.

As a result of early or late radiation damage, but more commonly early, mural or occluding hyaline thrombi may develop, resulting in organization.

In addition to the changes in the vessel wall proper there may be marked endothelial proliferation, even leading to obliteration, in the vasa vasorum.

Characteristic as these vascular lesions due to radiation in larger vessels may appear, they are not entirely specific. At times, as for example in the cervix or the uterus in later life, the normal involutional vascular changes may simulate a radiation reaction. In this instance an elastic tissue stain may be helpful, as the elastica is usually intact in the nonirradiated vessel and distorted or absent in the irradiated one. Vessels damaged as a result of frostbite or the injection of sclerosing solutions may also be confused.

Instead of using the conventional section method, Efskind peeled off and flattened portions of arterial intima for examination before and after the use of radiation and compared their appearance. Rabbits received a single dose of 2,500 r at 170 kilovolts and were killed at intervals of one hour to sixty days. After twenty-four hours the endothelial nuclei showed a tendency to palisade arrangement, and in two days they became vacuolated. Abnormal mitoses did not occur. Applying similar methods of examination in 5 cases in which patients received 3,500 to 4,500 r in divided doses (at 170 kilovolts with a 1 mm. copper filter), he found multinucleate cells due to mitosis without cytoplasmic cleavage. The endothelium of the larger vessels he found to have greater resistance.

Instances of actual perforation of the aorta have been reported usually following radium therapy for carcinoma of the esophagus (Cade, p. 161).

BIBLIOGRAPHY

THE HEART

Ajisaka, S.: Jap. J. Obst. & Gynec. **19**:430, 1936.
Bartsch, H., and Wachner, G.: Klin. Wchnschr. **16**:743, 1937.
Borman, M. C.: Am. J. Roentgenol. **18**:111, 1927.
Davis, K. S.; Radiology **3**:301, 1924.
Desjardins, A. U.: Am. J. Roentgenol. **27** (supp.):495, 1932.
Emery, E. S., Jr., and Gordon, B.: Am. J. M. Sc. **170**:884, 1925.
Engelmann: Fortschr. a. d. Geb. d. Röntgenstrahlen (Kongresshft.) **50**:40, 1934.
Faust, H.: Strahlentherapie **43**:749, 1932.
Freid, J. R., and Goldberg, H.: Am. J. Roentgenol. **43**:877, 1940.
Gabriel, G.: Strahlentherapie **34**:813, 1929.
Gendreau, J. E.: Ann. Surg. **93**:476, 1931.
Goldhaber, G., and Back, A.: Proc. Soc. Exper. Biol. & Med. **48**:150, 1941.
Gordon, B.; Shoup, B. F., and Emery, E. S., Jr.: Am. J. Roentgenol. **11**: 328, 1924.
Gottesman, J., and Bendick, A. J.: Am. J. M. Sc. **171**:715, 1926.
Granzow, J.: Arch. f. Gynäk. **151**:612, 1932.
Hartman, F. W.; Bolliger, A.; Doub, H. P., and Smith, F. J.: Bull. Johns Hopkins Hosp. **41**:36, 1927.
Karlin, M. I., and Mogilhitsky, B. N.: Frankfurt. Ztschr. f. Path. **43**:434, 1932.
Krebs, A.: Ztschr. f. Kreislaufforsch. **28**:701, 1936.
Leach, J. E., and Sugiura, K.: Am. J. Roentgenol. **45**:414, 1941.
Levy, R. L. ,and Golden, R.: Am. J. M. Sc. **194**:597, 1937.
Lucarelli, G.: Tumori **9**:85, 1935.
Oana, M.: Jap. J. Exper. Med. **10**:73, 1932.
Parade, G. W.: Med. Klin. **31**:1396, 1935.
Renfer, E.: Cancer **4**:431, 1927.
Ross, J. M.: J. Path. & Bact. **352**:899, 1932.
Schweizer, E.: Strahlentherapie **18**:812, 1924.
Thibaudeau, A. A., and Mattick, W. L.: J. Cancer Research **13**:251, 1929.
Toyoma, T.: Tohoku J. Exper. Med. **20**:591, 1933.
Tsuzuki, M.: Am. J. Roentgenol. **16**:134, 1926.
Warren, S. L., and Whipple, G. H.: J. Exper. Med. **35**:187, 1922.
Warthin, A. S., and Pohle, E. A.: Arch. Int. Med. **43**:15, 1929; Am. J. Roentgenol. **25**:635, 1931.
Zwaardemaker, H., and Feenstra, T. P.: Strahlentherapie **36**:368, 1930.

THE BLOOD VESSELS

Baermann, G., and Linser, P.: München. med. Wchnschr. **51**:996, 1904.
Bagg, H. J.: Am. J. Anat. **30**:133, 1922.
Cade, C.: Malignant Disease and Its Treatment by Radium, Baltimore, Williams & Wilkins Company, 1940, pp. 145 and 161.
Cramer, H.: Strahlentherapie **28**:431, 1928.
Daland, E. M.: Surg., Gynec. & Obst. **72**:373, 1941.
David, O., and Gabriel, G.: Strahlentherapie **15**:1, 1923.
Dobrovolskaia-Zavadskaia, N. A.: Lyon chir. **21**:397, 1924.
Downing, V.; Bishop, F. W., and Warren, S. L.: Am. J. Roentgenol. **43**:249, 1940.
Efskind, L.: Acta path. et microbiol. Scandinav. **17**:481, 1940.
Ellinger, F.: The Biologic Fundamentals of Radiation Therapy, translated by R. Gross, New York, Nordeman Publishing Company, 1941, p. 87.

Fabry, J.: Med. Klin. **22**:1408, 1926.
Failla, G.; Quimby, E. H., and Dean, A.: Am. J. Roentgenol. **9**:479, 1922.
Flaskamp, W.: Ueber Roentgenschäden und Schäden durch radioaktive Substanzen, in Meyer, H.: Sonderbände zur Strahlentherapie, Berlin, Urban & Schwarzenberg, 1930, vol. 12, p. 1.
Gassmann, A.: Fortschr. a. d. Geb. d. Röntgenstrahlen **2**:451, 1899; Arch. f. Dermat. u. Syph. **70**:97, 1904.
Gudernatsch, J. F., and Bagg, H. J.: Proc. Soc. Exper. Biol. & Med. **17**:183, 1920.
Lazarew, N. W., and Lazarewa, A.: Strahlentherapie **23**:41, 1926.
Linser, P.: Fortschr. a. d. Geb. d. Röntgenstrahlen **8**:97, 1904.
MacKee, G. M.: X-Rays and Radium in the Treatment of Diseases of the Skin, ed. 2, Philadelphia, Lea & Febiger, 1927, p. 319.
Porter, C. A.: J. M. Research **21**:357, 1909.
——and White, C. J.: Ann. Surg. **46**:649, 1907.
Ricker, G.: Strahlentherapie **5**:679, 1915.
Rudis-Jicinsky, J.: New York M. J. **76**:850, 1902.
Unna, P. G.: Fortschr. a. d. Geb. d. Röntgenstrahlen **8**:67, 1904.
Wetterer, J.: Handbuch der Röntgentherapie, Leipzig, O. Nemnich, 1908.
Windholz, F.: Strahlentherapie **59**:662, 1937.
Wolbach, S. B.: J. M. Research **21**:415, 1909.

VII. EFFECTS OF RADIATION ON THE URINARY SYSTEM

THE KIDNEYS AND URETERS

Much interest in the effect of radiation on the kidneys has been aroused in recent years because of the use of roentgen therapy of high voltage for renal tumors.

The maximum amount of treatment that may be safely delivered to the region of the kidney without irreparable damage to the skin and intervening tissues has been stated as 5 erythema doses, given in divided doses of 300 r each, three large fields being used (Dean). This is a total of about 7,200 r to the skin, generated at 200,000 volts, with a target-skin distance of 70 cm. and filtration through 0.5 mm. of copper. Many of the patients whose cases have been reported received considerably less than this amount, as have most of the animals irradiated experimentally. However, in regard to animals it should be remembered that the relatively small amount of tissue intervening between the kidneys and the skin means that a much greater proportion of the dose to the skin is effective than in the human being.

The kidneys are moderately responsive to radiation but should not be considered as radiosensitive. According to Desjardins, they are less sensitive than lymphoid tissue, skin or the liver, but more sensitive than muscle and nerve tissue. Doub, Hartman and Bolliger regarded the kidneys as among the most susceptible organs as far as anatomic changes and loss of function are concerned, acute changes developing in response to radiation more readily than in the spleen, the liver or the ovaries and less readily than in the intestine or the adrenals. They pointed out that by direct irradiation of the exposed kidney nephritis with hypertension could be produced both clinically and experimentally

and advised avoiding irradiation of the renal areas in young persons. They mentioned 16 cases of nephritis which developed or became apparent during or after high voltage roentgen therapy.

No renal damage was noted by Whipple and co-workers [1] even in dogs they had irradiated heavily. Elward and Belair regarded the epithelium of the kidneys as less sensitive to radiation injury than that of the skin, the lung or the liver but as more sensitive than either endothelial cells or connective tissue cells. The oxygen consumption of isolated renal tissue is not affected by 30,000 r generated at 200 kilovolts nor by 4,164 millicurie hours of gamma radiation from radon, although gamma and beta radiation totaling 4,000 to 6,500 millicurie hours do reduce it (Goldfeder and Pershing).

Tubular epithelium growing in tissue culture requires 25,000 r or more at 200 kilovolts for any definite change to be produced. The epithelium of the distal tubules is specially resistant, as are unorganized masses of renal epithelium. Secretory activity greatly increases the sensitivity (Chambers and Cameron).

Impiombato considered the kidneys as relatively insensitive organs. He irradiated rabbits with single doses of 500, 1,000, 2,000 or 3,000 r each and examined the kidneys microscopically from three to forty-five days afterward. The animals receiving 3,000 r died fairly promptly of hepatic and intestinal lesions. Doses of less than 2,000 r (7 by 7 cm. field, 40 cm. spark gap, 2 milliamperes) had practically no effect. More than 2,000 r (three times the erythema dose) caused renal hyperemia and congestion, then degeneration of epithelial cells (a direct effect rather than one due to vascular damage) and finally secondary fibrosis.

Gabriel, using a wide range of dosage in irradiation of exposed kidneys, noted atrophy and hyalinization of the glomeruli with secondary tubular change. He felt that the effect was primarily exerted on the blood vessels of the kidney and that those of the medulla were the first to be affected. The smaller doses of roentgen rays produced vasodilatation; the larger ones caused constriction. His experiments were carried out on cats, dogs and rabbits.

Atrophy of directly exposed kidneys was produced in rabbits by 2 to 10 erythema doses of roentgen rays (O'Hare and associates). One day after the radiation treatment was started, slight edema was present; three or more months later the kidneys were half normal size, the tubules largely destroyed, the glomeruli sclerosed and the vessels thickened.

Hartman and co-workers,[2] using dogs, heavily irradiated the abdomen over the renal area at 200 kilovolts. The earlier lesions were marked hyperemia, degeneration of tubular epithelium and some hemorrhage and infiltration by leukocytes. Later, the renal tubular epithelium

1. Hall and Whipple. McQuarrie and Whipple.
2. Hartman. Hartman, Bolliger and Doub (1926, 1927).

tended to disappear, and there was interstitial proliferation; the glomeruli showed thickening of Bowman's capsule and varying degrees of hyalinization; the vascular walls were thickened, suggesting endarteritis. According to Domagk and Enger and Preuschoff, the epithelium of the renal tubules, particularly of their proximal convoluted portion, is more susceptible to radiation injury than is that of the glomeruli. However, Domagk reported a case of true glomerular nephritis produced in a child by radiation directed to the abdomen.

Munger reported a group of cases in which a hypernephroma of one kidney was treated by radiation and the other kidney as well as the other viscera were shielded. In 1 case the other kidney, nephritic to begin with, showed depression of function following the treatment but recovered. In another case the excretion of phenolsulfonphthalein was depressed to 35 per cent in two hours but returned to normal after ten days. Munger expressed the belief that in some of these cases in which as much as 8,400 r at 650 kilovolts was administered the radiation change was less than would be expected following radiation at 200 kilovolts. One of his cases is exceptionally interesting in that the renal tissue persisting adjacent to the tumor of the kidney showed after operation, without any treatment by radiation, mild tubular and glomerular changes not unlike those which some investigators have ascribed to injury by radiation. Furthermore, radiation treatment was carried out later over the site of the renal tumor with the remaining kidney shielded. The function of the latter remained normal and analysis of the urine gave negative results.

Hagner and Coleman reported the case of a 45 year old man who received four roentgen treatments of 300 milliampere minutes each at 70 cm. distance for a tumor of the left kidney. The rest of the viscera in the field were screened. Some radiation sickness developed. Twenty-four days after the last treatment a primary tumor diagnosed as renal cell carcinoma was removed with the left kidney. On the tenth day after operation there was complete suppression of urine and the man died in uremia. Since the remaining kidney was screened during the radiation therapy and the amount of radiation was moderate, this could hardly be a roentgen ray effect.

Waters, regarding preoperative irradiation of cortical renal tumors, stated that he used total doses of 1,600 to 3,500 r at 200 kilovolts, given at the rate of 195 to 345 r a day by three to four portals. This dosage would usually reduce the size of the tumor about one half. The other kidney was screened and was unaffected except in 3 cases in which slight damage occurred, followed by good regeneration.

Willis and Bachem, using dogs, gave 3 to 7 erythema doses at 140 kilovolts directly to the kidney by means of a lead cone. They found primary tubular degeneration followed by obliteration of capillaries, scarring, and atrophy of the glomeruli.

The first degenerative changes appear in the renal tubular epithelium (Earlam and Bolliger), especially that of the convoluted tubules (Enger and Preuschoff; Liberson and Vail). Warthin felt on the basis of observations of irradiated rats and mice that the degeneration was not due directly to the effect of radiation. This belief has not been substantiated.

When the whole body or the epigastrium of a rabbit is exposed to roentgen radiation at 200 kilovolts, killing the animals in three to nine days, slight renal effects appear—congestion and cloudy swelling of the cortex, free blood within Bowman's capsule and desquamated tubular cells forming granular casts (Kolodny).

Tsuzuki noted that 32 per cent of an erythema dose produced hyperemia of the glomeruli; 48 per cent produced degeneration of the tubular epithelium at the end of forty-eight hours, which returned to normal in ninety-six hours. More marked tubular degeneration, due to heavier radiation, is followed by fibrosis; thickening of blood vessel walls develops next, and then atrophy and hyalinization of the glomeruli develop (Hartman).

The late changes from radiation produce in the kidney of the dog shrinkage and adherence to surrounding tissue. The cortex is somewhat more contracted than is the medulla. Microscopically, the glomeruli are identifiable but small and atrophic. Some thickening and hyalinization of Bowman's capsule appear. The tubular epithelium has virtually disappeared, leaving much hyalinized interstitial connective tissue, and here and there scattered tubules with distorted epithelial cells occur. The renal capsule is thickened and hyalinized (Earlam and Bolliger).

Following the injection of radium chloride to produce chronic radium poisoning, acute tubular nephritis with focal calcification has occurred. The convoluted tubules and Henle's loops were most involved (Thomas and Bruner).

The first work on the subcutaneously transplanted kidney was done by Schulz and Hoffmann in 1905, who gave 5 erythema doses and noted degeneration of the epithelial cells in ten days and proliferation of interstitial cells in twenty-one days. The primary effect was tubular degeneration; they regarded the glomerular changes as secondary.

Page transplanted each kidney to a site beneath the skin of the loin and irradiated it by repeated small doses, totaling 2 erythema doses to each kidney, to produce experimental nephritis in dogs. The radiation was given after a three week control period. A current strength of 130 kilovolts, 5 milliamperes, filtered through 3 mm. of aluminum, was employed at 30 cm. distance. Four to six days later the dogs showed protein casts and red cells in the urine and later died in uremia. At autopsy the kidneys were found to be shrunken with scarred surfaces, adherent capsules and thin cortices. There was hyaline necrosis of

Henle's loops with proliferation of new epithelium. There was occlusion of the smaller blood vessels, so that fibrosis occurred. The capillaries and glomerular walls were thickened and became more cellular.

The early renal changes due to radiation may be summarized as hyperemia and swelling and desquamation of the epithelium of the tubules, especially of the convoluted tubules. Later gross shrinkage occurs, with capsular thickening. Microscopically, marked tubular degenerative changes persist, with slight attempt at regeneration. The glomeruli, although quite resistant, show varying degrees of hyalinization. The blood vessels are not greatly damaged but may show some endarteritic alteration. The interstitial tissue becomes much condensed and hyalinized.

The ureters are fairly resistant, but occasionally in heavy irradiation of the pelvic region stenosis of these structures is produced as a result of fibrosis of their walls associated with a greater or lesser degree of epithelial injury.

BLADDER

The chief interest in reactions of the bladder to radiation has related to the cystitis following therapeutic irradiation of the pelvic region. Few experimental data are available. The vesical epithelium is moderately resistant, that of the human being withstanding doses up to 8,400 r (measured in air) at 200 or 400 kilovolts in divided doses when, for example, a patient is treated for carcinoma of the cervix by cross firing. To be sure, hyperemia and some desquamation and swelling of epithelium are produced, but as a rule permanent damage does not result.

Three stages of response to radiation on the part of the bladder may be recognized: (1) a primary erythema due to hyperemia, appearing in twenty-four hours or less, (2) a secondary erythema due also to hyperemia, appearing in three to four weeks, and (3) a late reaction due to vascular occlusion and connective tissue changes, rarely appearing before one year after radiation (Dean).

According to Schmitz, the frequency of the late changes as a sequel of treatment for cervical cancer is slight (4 among 371 cases, according to Newell and Crossen), but if doses of radiation greater than 3,500 millicurie hours are given, late changes are apt to occur (Dean). Some of them may take years to appear—in one instance of heavy treatment for carcinoma of the cervix a seven year period elapsed before a vesicovaginal fistula developed (Rulle).

The early stages of reaction comprise varying degrees of hyperemia, vascular injury and edema, and desquamation of epithelial cells, differing but little from the findings in other mucous membranes. The late reaction appears grossly as fissuring or ulceration of a pale or white mucosa with a few dilated blood vessels.

The ulcers are usually crusted with urinary salts and are often indistinguishable by inspection from carcinoma. Microscopically, the hyalinized connective tissue, the thick-walled or dilated blood vessels and the fibrosis of the muscularis are quite characteristic. Giant and bizarre fibrocytes are often seen. These, however, are not pathognomonic, as they may be seen in giant cell cystitis.

BIBLIOGRAPHY

THE KIDNEYS AND URETERS

Bolliger, A., and Laidley, J. W. S.: M. J. Australia **1**:136, 1930.
Chambers, R., and Cameron, G.: Radiology **37**:186, 1941.
Dean, A. L.: J. Urol. **37**:692, 1937.
Desjardins, A. U.: J. A. M. A. **83**:109, 1924.
Domagk, G.: Beitr. z. path. Anat. u. z. allg. Path. **77**:525, 1927.
Doub, H. P.; Hartman, F. W., and Bolliger, A.: Radiology **8**:142, 1927.
Earlam, M. S. S., and Bolliger, A.: M. J. Australia **1**:826, 1932.
Ellinger, F.: The Biologic Fundamentals of Radiation Therapy, translated by R. Gross, New York, Nordeman Publishing Company, 1941, p. 51.
Elward, J. F., and Belair, J. F.: Radiology **33**:450, 1939.
Enger, R., and Preuschoff, P.: Arch. f. path. Anat. u. Physiol. **283**:489, 1932.
Gabriel, G.: Strahlentherapie **22**:107, 1926.
Goldfeder, A., and Pershing, J. L.: Radiology **31**:81, 1938.
Hagner, F. R., and Coleman, S. R.: Tr. Am. A. Genito-Urin. Surgeons **26**: 173, 1933.
Hall, C. C., and Whipple, G. H.: Am. J. M. Sc. **157**:453, 1919.
Hartman, F. W.: Ann. Clin. Med. **5**:599, 1927.
——Bolliger, A., and Doub, H. P.: Am. J. M. Sc. **172**:487, 1926; J. A. M. A. **89**:1936, 1927.
Impiombato, G.: Radiol. med. **22**:487, 1935.
Kolodny, A.: Am. J. Path. **1**:285, 1925.
Liberson, J. G., and Vail, S. S.: Ann. de roentgenol. et radiol. **2**:248, 1927.
McQuarrie, I., and Whipple, G. H.: J. Exper. Med. **35**:225, 1922.
Munger, A. D.: J. Urol. **37**:680, 1937.
O'Hare, J. P.; Altnow, H.; Christian, T. D.; Calhoun, A. W., and Sosman, M. C.: Boston M. & S. J. **194**:43, 1926.
Page, I. H.: Am. J. M. Sc. **191**:251, 1936.
Schulz, O. E., and Hoffmann, R. S.: Deutsche Ztschr. f. Chir. **79**:350, 1905.
Thomas, H. E., and Bruner, F. H.: Am. J. Roentgenol. **29**:641, 1933.
Tsuzuki, M.: Am. J. Roentgenol. **16**:134, 1926.
Warthin, A. S.: Am. J. M. Sc. **133**:736, 1907.
Waters, C. A.: Am. J. Roentgenol. **33**:149, 1935.
Willis, D. A., and Bachem, A.: Am. J. Roentgenol. **18**:334, 1927.

THE BLADDER

Dean, A. L.: J. A. M. A. **89**:1121, 1927.
Newell, Q. U., and Crossen, H. S.: Surg., Gynec. & Obst. **60**:763, 1935.
Rulle, P. H.: Zentralbl. f. Gynäk. **53**:357, 1929.
Schmitz, H.: Am. J. Roentgenol. **24**:47, 1930.
Stacy, L. J.: Am. J. Roentgenol. **19**:323, 1928.

Notes and News

Fellowships in Cancer Research.—The Finney-Howell Research Foundation, Inc., announces that all applications for fellowships in cancer research for next year must be on hand by Jan. 1, 1943. Applications received after that date cannot be considered for 1943 awards, which will be made March 1, 1943. Fellowships carrying an annual stipend of $2,000 are awarded for the period of one year, with the possibility of renewal up to three years. When deemed wise by the directors, special grants of limited sums may be made to support the work carried on under a fellowship. Applications must be made on the blank form furnished by the secretary, 1211 Cathedral Street, Baltimore, Md.

Prize for Cancer Research.—The Memorial Hospital for the Treatment of Cancer and Allied Diseases, in New York, has awarded the Katherine Berkan Judd prize of $1,000 for "the most outstanding research of the year in the field of cancer" to Charles B. Huggins, professor of urology at the University of Chicago, in recognition of his work on the relationship of testosterone to carcinoma of the prostate.

Book Reviews

Blood Grouping Technic. A Manual for Clinicians, Serologists, Anthropologists, and students of Legal and Military Medicine. By Fritz Schiff, M.D., late chief of the department of bacteriology of Beth Israel Hospital, New York, and William C. Boyd, Ph.D., associate professor of biochemistry at the Boston University School of Medicine and associate member of the Evans Memorial of the Massachusetts Memorial Hospitals, Boston. With a foreword by Karl Landsteiner, of the Rockefeller Institute for Medical Research. P. 248, with 45 illustrations. Price $5. New York: Interscience Publishers, Inc., 1942.

Schiff, whose untimely death interrupted a distinguished career devoted largely to the study of blood groups, was the author of a widely known small German manual on the technic of blood grouping (Die Technik der Blutgruppenuntersuchung, third edition, Berlin, Julius Springer, 1932). In the present book the material has been revised, brought up to date and considerably expanded by the addition of much that carries the imprint of the work of Boyd.

The theoretic foundations of blood groups are presented in a brief introductory chapter. The five following chapters deal with the general technic of grouping (sixty-five pages), the application to blood transfusions (thirty-nine pages), the exclusion of paternity (thirty-two pages), the grouping of blood stains and of tissues other than blood (twenty-eight pages), the blood groups in animals (ten pages) and applications in anthropologic studies (forty-five pages). All these phases are adequately presented. Sources of error are given special consideration. The more important references are included.

The reviewer finds it difficult to agree with the statement on page 123 that "the use of universal donors and universal recipients as such has been practically abandoned," and with the statement on page 130 regarding the use of the numerical classifications of blood groups: "These earlier systems of naming are still unfortunately used to some extent in some hospitals." It is regrettable but the more correct statement would have been that universal donors are frequently used and that the "earlier systems of naming" are used in many hospitals.

The translation of the German text is very satisfactory, with extremely rare lapses like this one: "The sera from the persons being tested are reacted with A and B cells. . . ." (page 143). The absence of typographic errors indicates careful editing.

The chapters and their subdivisions are numbered according to the so-called decimal system, which is used widely in libraries for cataloging books. Librarians find it useful, but it may be questioned whether it will be found worth while for the contents of a small monograph. It is a little cumbersome to use for reference a number consisting of six digits and a period—for instance, 3.16432. It seems that even the authors found it so, for in the subject index the old-fashioned way of referring to page numbers is followed.

The monograph is recommended to all interested in the subject.

Synopsis of Pathology. W. A. D. Anderson, M.A., M.D., assistant professor of pathology, St. Louis University School of Medicine; pathologist, St. Mary's Group of Hospitals. Pp. 661, with 294 illustrations and 17 color plates. Price $6. St. Louis: The C. V. Mosby Company, 1942.

This synopsis covers well the field of textbooks of pathology. The first eleven chapters deal with general pathology as customarily understood and with such new subjects as vitamin deficiencies and virus-rickettsial infections. The other fourteen chapters take up pathology by systems, essentially from the morphologic side, except those of the eye, ear, nose and larynx. The presentation is condensed

—synoptic—but clear, sound and competent, with a number of helpful factual summaries. At the ends of the chapters are listed well selected recent articles and reviews in easily available publications. Almost without exception, the many illustrations, gross and microscopic, are commendable and will serve their purpose well. The seventeen plates in color are taken from other books issued by the publisher of this book. A few comments may be ventured. Glanders is not mentioned, nor are the blood groups. "Flecked spleen" or "speckled spleen" is a better term than *Speckmilz,* which is not listed in Dorland's "American Illustrated Medical Dictionary." The excellent chapter on virus and rickettsial diseases by Henry Pinkerton makes no mention of the entrance of the virus of poliomyelitis by the digestive tract, with the bearing of this on prevention. Is the value of immune serum in the preparalytic stage as definitely established as stated? In the paragraphs on the response of cancer to radiation the role of the extent of the cancer, particularly squamous carcinoma, in the resistance to radiation is not considered. This is a frequent mistake. In the early stages squamous carcinoma of the larynx, mouth and other accessible sites may be curable by adequate radiotherapy. Virus sarcoma as such cannot be transferred or transmitted by cell-free filtrate, but it may be incited de novo by the virus in the filtrate. Metastasis by implantation can take place on mucous as well as on serous surfaces, as illustrated by the implantation of carcinoma of the esophagus on the gastric mucosa. The discussion of tumors would have been more compact and to the point had the words "cancer," "cancerous" and "noncancerous" been used as consistently as possible in place of "malignancy," "malignant" and "benign." Such terms as "benign prostatic enlargement" are at best awkward and unscientific. Why should venereal warts and pyogenic granuloma be listed with tumors? These are questions and suggestions to be considered in the preparation of a new edition, which no doubt will be called for now that the period of required medical study has to be contracted. Anderson's synopsis will be of direct service in the adjustment of the medical curriculum to the new conditions.

Medical Parasitology. James T. Culbertson, assistant professor of bacteriology, College of Physicians and Surgeons, Columbia University. Pp. xvi + 285. Price $4.25. New York: Columbia University Press, 1942.

This book deals with the animal parasites that cause human diseases. The first part is devoted to a general consideration of infection, epidemics, resistance and immunity, diagnosis, treatment and prophylaxis in relation to these diseases. The second part takes up the diseases in question systematically in the following order: Protozoan infections (amebiasis, leishmaniasis, trypanosomiasis, intestinal flagellate infections, malaria, coccidiosis, ciliate infections), trematode and cestode infections (flatworms), and nematode infections (roundworms). There are two chapters on the arthropods (insects, crustaceans, etc.) of medical importance. Special technical methods are described in an appendix. The presentation is clear, orderly, compact. The book is well illustrated with twenty-one plates, each with several figures, and also with separate figures in the text. There are useful tables showing the routes of infection and the chief effects of animal parasites in man, the geographic distribution of parasitizations, diagnostic procedures, arthropods of medical importance, and other topics. The author has succeeded admirably in his purpose "to supply a small book useful chiefly to medical students and medical practitioners" about animal parasites of medical significance. Needless to say, under present conditions these agents and the diseases they cause will require increasing attention.

Books Received

ATLAS OF OPHTHALMIC PATHOLOGY PREPARED AT THE ARMY MEDICAL MUSEUM OFFICE OF THE SURGEON GENERAL U. S. ARMY FROM MATERIAL IN THE REGISTRY OF OPHTHALMIC PATHOLOGY. Major Elbert DeCoursey, Medical Corps, United States Army, former pathologist to the Registry; Colonel J. E. Ash, Medical Corps, United States Army, curator; Roy M. Reeve, photographer; Helen Campbell Wilder, microscopist; Lawrence P. Ambrogi, technician. Third edition, revised. Pp. 136. Price $5. Omaha: The American Academy of Ophthalmology and Otolaryngology, 1500 Medical Arts Bldg., 1942.

ATLAS OF OTOLARYNGIC PATHOLOGY PREPARED AT THE ARMY MEDICAL MUSEUM OFFICE OF THE SURGEON GENERAL UNITED STATES ARMY LARGELY FROM MATERIAL IN THE REGISTRY OF OTOLARYNGIC PATHOLOGY. Colonel J. E. Ash, Medical Corps, United States Army, Curator, with the assistance of Major J. L. Bernier, Dental Corps, United States Army, assistant pathologist; Roy M. Reeve, photographer. Third edition, revised. Pp. 173. Price $5. Omaha: The American Academy of Ophthalmology and Otolaryngology, 1500 Medical Arts Bldg., 1942.

WAR MEDICINE, A SYMPOSIUM. Editor, Commander Winfield Scott Pugh, Medical Corps, United States Navy, retired, formerly surgeon, City Hospital, New York; associate editor, Edward Podolsky, M.D.; technical editor, Dagobert D. Runes, Ph.D. Pp. 563. Price $7.50. New York: Philosophical Library, 1942.

SYNOPSIS OF PATHOLOGY. W. A. D. Anderson, M.D., assistant professor of pathology, St. Louis University School of Medicine; pathologist, St. Mary's Group of Hospitals. Pp. 661, with 294 illustrations, 17 color plates. Price $6. St. Louis: The C. V. Mosby Company, 1942.

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Subject entries are made for all articles. Author entries are made for original articles. Book Reviews and Obituaries are indexed under these headings in their alphabetical order under the letters B and O, respectively.